A METHOD OF INDUCING A CTL RESPONSE

CROSS REFERENCE

This application is a continuation-in-part of U.S. Patent Application No. 09/380,534, filed September 1, 1999, which was based on PCT Application No. PCT/US98/14289, filed July 10, 1998, which claimed priority from U.S. Patent Application No. 08/988,320, filed December 10, 1997, and from Canadian Patent Application No. 2,209,815, filed July 10, 1997, all of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The invention relates to a method of inducing a CTL response to an antigen by sustained, regular delivery of the antigen to an animal so that the antigen reaches the lymphatic system.

BACKGROUND OF THE INVENTION

Cytotoxic T lymphocytes (CTL) are white blood cells found in the blood, spleen and lymph. CTL have the ability to attack and kill other cells of the body in a highly specific manner. When CTL are stimulated by specific antigen, they migrate through the tissues of the body on a "search and destroy" mission for cells bearing the specific antigen. Whether of viral origin or tumor associated, CTL detect antigen that is bound to major histocompatability complexes (MHC) on the surface of potential target cells. Once CTL have identified the antigen on the cell surface, their function is to deliver a lethal hit to the cell.

Although there are hundreds of millions of CTL that reside in the spleen, each individual CTL exclusively responds to a unique and specific antigen. These individual CTL, dubbed CTL precursors (CTLp), undergo cell division or proliferate upon activation by specific antigen to produce daughter cells with precisely the same antigen specificity as the parent cell. This proliferation increases the total number, and thus the frequency, of that specific CTLp in the body. A proportion of these newly generated CTL briefly recirculate through the body (termed effector CTL), and have the ability to

identify and destroy cells bearing the specific antigen which they recognize. A significant body of experimental evidence suggests that CTL specific for tumor antigens can inhibit tumor growth. Unfortunately, most tumors have only a very weak capacity to stimulate CTL responses and there has been no means of inducing a CTL response then sustaining it over a period of time sufficient to continuously inhibit tumor growth. While many attempts to directly increase the capacity of tumor cells to stimulate tumor-clearing CTL responses in patients have been made, such attempts have met with limited success. Technical advances over the past ten years have, however, enabled the identification of natural peptide antigens that are present on tumor cells and which are recognized by CTL. These antigen targets include proteins expressed in significant overabundance, abnormally expressed embryonic proteins, protein products from mutated oncogenes or suppressor genes, or proteins derived from cancer-causing viruses present in tumor cells. The challenge has been to find a way in which to administer an antigen so that it induces an antitumor CTL response and maintains it over time. While many attempts have now been made to use these antigens clinically in a vaccine, the results have been less than satisfactory.

An explanation of why CTL therapies have been largely ineffective at eradicating or controlling tumors in a clinical setting include the following:

(a) Vaccine designs have been inadequate at initiating strong CTL responses;

(b) Tumor cells can down regulate MHC molecules, resulting in the loss of antigen presentation from the surface of cells, thereby escaping detection by CTL;

(c) After induction, effector CTL recirculation through the body is highly transient;

(d) After recirculation, CTL return to the spleen where they reside in a nonactive or resting state, and an increase in the numbers of CTLp residing in the spleen does not reflect active CTL immunity;

(e) In the case of tumors, regrowth of residual tumor cells following immunization goes undetected by CTLp residing in spleen in a "resting" state;

(f) Because CTL-stimulating antigen presenting cells (APC are targeted for destruction by the same CTL that they have activated, the CTL response is self-limiting, which precludes, under normal circumstances, the continuous stimulation for a long-lived CTL response.

A growing repertoire of tumor associated antigens are being discovered that are recognized by CTL. A variety of techniques have been suggested to render these antigens effective in CTL vaccines. These include immunization using synthetic peptide antigens mixed with an immunostimulatory adjuvant, such as the bacterial toxin BCG; inununization with multiple antigenic pcptide systems (MAPS), immunization with "professional" antigen presenting cells, which are isolated from the patient, pulsed with peptide antigen and inoculated back into the patient as a vaccine; immunization with peptides designed to stimulate both CTL and T helper cell populations; immunization with viruses or bacteria engineered to express tumor antigens; and immunization with polynucleotide expression vectors (so called DNA vaccines). Unfortunately, none of these approaches has been an unqualified success, As discussed above, the lack of vigorous therapeutic effects with these vaccine platforms reflects at least to some degree problems associated with inducing a strong initial CTL response and with maintaining ongoing "active" CTL immunity.

Studies by Glenny during the first quarter of the century revealed that aluminum compounds could enhance the strength of diphtheria vaccines. This was ostensibly the first of a long history of observations supporting a "depot" theory of immunization, which postulates that antigen slowly leaking into the tissues over an extended time correlates with the antigenic potency of a vaccine. Today, this antigen depot paradigm forms the intellectual backdrop to most adjuvant development programs. In one form or another, depot type adjuvants are intended to prolong the course of antigen delivery, by forming a lesion at the site of injection, or simply by the slow degradability of the adjuvant itself, which mixed with the specific antigen forms a depot at the site of injection. A second function generally attributed to adjuvants are their immunostimulatory effects, which appears to trigger the immune system to respond to the vaccine. However, adjuvants are a double-edged sword. They have inherent toxicities. But it is a feature of these toxicities that achieves a desired immunostimulatory and/or depot effect. Side effects such as tissue damage and granulornatous reaction at the site of injection, fever, and in some cases systemic reactions, such as Reiter's syndrome-like symptoms, uveitis and arthritis, are some of the risks associated with the use of adjuvants. Currently, the only adjuvant approved by the FDA is alum. It is relatively safe but does have side effects such as erythema,

subcutaneous nodules, contact hypersensitivity, and granulornatous inflammation. More importantly, alum only acts to potentiate a limited number of antigens, and it very predominantly stimulates humoral antibody responses rather than CTL immunity. Thus so far adjuvants have proved to be very ineffective components for vaccines aimed at inducing clinically relevant CTL responses.

Recent attempts to induce CTL responses using dendritic cells or other antigen presenting cells, despite being cumbersome, have shown some promise. New recombinant virus or bacterial systems carrying genes for specific antigen are effective at inducing primary CTL responses. The most effective viruses, for example, that induce strong CTL responses are those which replicate aggressively in the host. Yet because of the risk for serious or lethal complications as a result of infection, recombinant virus used in a cancer vaccine must be only weakly replicative, or be completely replication deficient. This trade-off between virulence and efficacy is at present an intractable problem.

DNA (or polynucleotide) vaccines are also being developed for the purpose of inducing CTL immunity. Once again, the system has intrinsic limitations that preclude its efficacy in inducing long-lasting CTL immunity. The DNA vaccines consist of a plasmid or similar genetic construct for expressing the antigen of interest. Uptake of the plasmid system by cells of the body results in expression of the antigen and induction of CTL. However, once cells expressing the construct have succeeded in inducing CTL, they are themselves targets for eradication by the CTL. The CTL inducing effect is thus again transient. Moreover, the polynucleotide vaccines have thus far suffered from poor efficiency in terms of CTL induction.

With difficulties in achieving strong primary and/or persisting CTL responses, there are a number of clinical trial groups now using repeated injections of cancer vaccines. The use of antigenically complex materials in the vaccine formulation, such as recombinant virus, or the costs associated with repetitive treatment using cultured APC will, however, make such an approach difficult. On the one hand, repetitive immunization with antigenically complex materials drives the immune system to elaborate a humoral antibody, as opposed to a CTL response, while on the other hand, use of a minimal CTL antigen (such as a nonamer peptide) which does not efficiently drive an antibody response, has also failed to induce a CTL response. Attempts to

develop adjuvants that enhance the immunostimulatory aspects of minimal CTL antigens have resulted in the production of materials (i.e. adjuvants) that also induce a competing humoral immune response, or, which simply offer little CTL stimulatory effect.

It has also been suggested that certain controlled release technology using microspheres or liposomes with subunit antigens and peptides might be effective to enhance immunogenecity. The combination of sustained release and depot effect is suggested to reduce the amount of antigen needed and eliminate booster shots. However, the preparation of such compositions is difficult and unpredictable, and vaccine formulations based on this technology have not been translated into effective clinical treatments.

As can be seen from the foregoing, there has been little success at developing a CTL vaccine that is both capable of inducing a strong CTL response then sustaining that response over time. The development of a vaccine with these capabilities is essential before effective anti-tumor therapy based on CTL immunity can be contemplated.

OBJECTS OF THE INVENTION

An object of this invention is to provide a method for inducing or sustaining a specific CTL immununological response in a mammal over time.

Another object of this invention is to provide a method for treating a mammal having a malignant tumor or infectious disease by inducing and sustaining an immunological attack on the malignant tumor or infectious disease in the mammal.

It is a further object of this invention to provide an article of manufacture useful for inducing and sustaining a specific immunological CTL response in a mammal over time.

It is a further object of this invention to provide an article of manufacture useful for treating a mammal having a malignant tumor or infectious disease, which article is designed to induce and maintain an immunological attack on the malignant tumor or infectious disease in the mammal.

It is a further object of this invention to provide a portable device for sustained delivery of an antigen to a mammal having a malignant tumor or infectious disease,

where the antigen stimulates the mammal's immune system to attack the tumor or infectious disease and the device is located outside the mammal.

It is still a further object of this invention to provide an implantable device for sustained delivery of an antigen to a mammal having a malignant tumor or infectious disease, where the antigen stimulates the mammal's immune system to attack the tumor or infectious disease.

It is a further object of this invention to provide antigen compositions and containers therefor that are useful in the methods, devices, and/or articles of manufacture of this invention.

Other objects of this invention may be apparent to those of skill in the art by reading the following specification and claims.

SUMMARY OF THE INVENTION

In one aspect of the invention, a method is provided for inducing an immunological CTL response to an antigen by sustained, regular delivery of the antigen to a mammal so that the antigen reaches the lymphatic system. In particular, the antigen is delivered to the mammal at a level sufficient to induce an immunologic CTL response in the mammal and the level of the antigen in the mammal's lymphatic system is maintained over time sufficient to maintain the immunologic CTL response. Preferably, the antigen is delivered directly to the mammal's lymphatic system, such as to the spleen, a lymph node or lymph vessel.

Also provided is a method of treating an animal having a disease, or being predisposed to a disease, to which the animal's immune system mounts a cell-mediated response to a disease-related antigen to attack the disease. In this aspect of the invention, a disease-matched antigen is delivered to the animal at a level sufficient to induce an increased CTL-response in the animal which is then maintained in the animal by sustained, regular delivery of the disease-matched antigen to the animal for a time sufficient to treat the disease. The sustained, regular delivery of the antigen is done in a manner that maintains the level of antigen in the animal's lymphatic system. Preferably, the sustained, regular delivery is achieved by pumping a physiologically-acceptable, composition of the antigen from a device held external of or implanted in the animal's body so that the antigen reaches the animal's lymph system. Optionally, a cytokine that

is capable of enhancing the CTL response is delivered and/or maintained along with the antigen. Diseases addressed in this manner include cancer and pathogenic diseases.

In a further aspect of the invention, an article of manufacture is provided for delivering an antigen that induces a CTL response in an animal. In particular, the article comprises a reservoir of a physiologically-acceptable, antigen-containing composition that is capable of inducing a CTL response in an animal; a pump connected to the reservoir to deliver the composition at a defined rate; a transmission line to discharge the composition from the reservoir; and, optionally, a delivery line connected to the transmission line, which delivery line is of a size suitable for positioning in the animal and for delivery of the composition in a manner that reaches the lymphatic system of the animal.

In a further aspect of the invention, a process is provided for preparing a system useful for inducing a sustained CTL response in an animal needing such a response, which comprises placing a physiologically-acceptable, antigen-containing composition in a reservoir having a pump for delivering the composition at a defined rate through a transmission line to the animal.

Another aspect of the invention is a method of inducing and/or sustaining an immunological CTL response in a mammal by delivering an antigen in the form of a polypeptide directly to the lymphatic system of the mammal. The antigen can be delivered at a level sufficient to induce an immunologic CTL response in the mammal and the level of the antigen in the mammal's lymphatic system is preferably maintained over time sufficient to maintain the immunologic CTL response.

The antigen can be an 8-10 amino acid peptide. Further, the peptide sequence can be derived from a tumor-associated antigen. Examples of tumor-associated antigens include MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein , ß-HCG, BCA225, BTAA, CA

125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, and the like.

The peptide sequence also can be derived from a microbial antigen. Further, the antigen can be provided as a component of a microorganism or mammalian cell. Examples of microorganisms include a protozoan, a bacterium, a virus, and the like; the mammalian cell can be an antigen presenting cell, such as, for example, a dendritic cell.

The antigen can be a native component of the microorganism or mammalian cell. The microorganism or mammalian cell can include, for example, an exogenous antigen. Also, the microorganism or mammalian cell can include a recombinant nucleic acid encoding or promoting expression of the antigen. The microorganism or mammalian cell can express a tumor-associated antigen, or a microbial antigen native to a second microbial species. The antigen can be provided as an 8-10 amino acid peptide.

The present invention in another aspect includes a method of inducing and/or sustaining an immunological CTL response in a mammal by delivering an antigen, in the form of a vector that can include a nucleic acid encoding the antigen, directly to the lymphatic system of the mammal. The antigen can be delivered at a level sufficient to induce an immunologic CTL response in the mammal and the level of the antigen in the mammal's lymphatic system is preferably maintained over time sufficient to maintain the immunologic CTL response.

The vector can be a plasmid and the like. The vector further can include a bacterium and the like. The bacterium, for example, can include *Listeria, Shigella, Salmonella*, *Escherichia*, and the like. The vector, for example, can be a virus, such as, for example, pox viruses, adenoviruses, adeno-associated viruses, retroviruses, herpesviruses, and the like.

The nucleic acid can encode, for example, a tumor-associated antigen. Examples of tumor-associated antigens include MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-

4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein , ß-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, and the like.

The nucleic acid can encode, for example, a microbial antigen. Examples of microbial antigens include a viral antigen, a bacterial antigen, a protozoal antigen, and the like. The nucleic acid can encode, for example, a protein or other polypeptide. The nucleic acid also can encode an 8-10 amino acid peptide.

The nucleic acid can be plasmid DNA in a formulation comprising about 1-10% ethyl alcohol, 0-1% benzyl alcohol, 0.25-0.5mM EDTA and a citrate-phosphate buffer of pH 7.4-7.8, comprising about 3-50mM citrate and about 90 –200mM phosphate. For example, the formulation can include 1% ethyl alcohol, 1% benzyl alcohol, 0.5mM EDTA and a citrate-phosphate buffer of pH 7.4 to 7.8 comprising 50mM citrate and 100mM phosphate.

The invention in another aspect provides a method of inducing and/or sustaining an immunological CTL response in a mammal by delivering a microorganism or mammalian cell directly to the lymphatic system of the mammal. The microorganism or mammalian cell are preferably delivered at a level sufficient to induce an immunologic CTL response in the mammal and the level of the microorganism or mammalian cell in the mammal's lymphatic system is preferably maintained over time sufficient to maintain the immunologic CTL response.

A further aspect of the invention is a method of inducing and/or sustaining an immunological CTL response in a mammal by delivering a nucleic acid, capable of conferring antigen expression, directly to the lymphatic system of the mammal. The nucleic acid can be delivered at a level sufficient to induce an immunologic CTL response in the mammal and the level of the nucleic acid in the mammal's lymphatic system is preferably maintained over time sufficient to maintain the immunologic CTL response.

A further aspect of the invention is a method of inducing and/or sustaining an immunological CTL response in a mammal by delivering a non-peptide antigen directly to the lymphatic system of the mammal. The antigen is preferably delivered at a level sufficient to induce an immunologic CTL response in the mammal and the level of the antigen in the mammal's lymphatic system is preferably maintained over time sufficient to maintain the immunologic CTL response.

The invention also provides an article of manufacture for delivering an antigen that induces a CTL response in an animal. In particular, the article can be an external device. The article can include a reservoir of a physiologically-acceptable, antigen-containing composition that can be capable of inducing a CTL response in an animal, a pump connected to the reservoir to deliver the composition at a defined rate, a transmission line to discharge the composition from the reservoir; and, a delivery line connected to the transmission line. The delivery line can include a catheter of at least 20mm for positioning in the animal and for delivery of the composition to the lymphatic system of the animal.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 is a graph showing the lysis of target cells by CTL versus the effector/target ratio when antigen is delivered as a single dose (circles) and when antigen is delivered by a continuous pump (triangles).

Figure 2 (A and B) are graphs showing the lysis of target cells by CTL versus the effector/target ratio when antigen is delivered as a single dose (circles), when antigen is delivered by a continuous pump (triangles) and negative control (squares) at (A) 36 hours and (B) 7 days.

Figure 2C is a graph showing the footpad swelling versus time when antigen is delivered as a single dose (circles) and when antigen is delivered by a continuous pump (triangles).

Figure 3 is a graph showing the lysis of target cells by CTL versus the dose of the peptide antigen when the antigen is delivered subcutaneously, intravenously and intrasplenically.

Figure 4 is a bar graph showing tritiated thymidine uptake in CTL cells induced by antigen introduced intravenously, intrasplenically and subcutaneously.

Figure 5 is a rough schematic of a human lymphatic system.

Figure 6. Comparison of anti-peptide CTL responses following immunization with various doses of DNA by different routes of injection.

Figure 7. Comparison of anamnestic antiviral CTL responses following immunization with various doses of DNA by different routes of injection.

Figure 8. Protective immunity against systemic and peripheral virus infection following intra-lymph node immunization with DNA. LCMV titer in spleen (A) and Vacc-G2 vaccinia titers in ovary (B) following indicated immunization and subsequent viral challenge.

Figure 9. Growth of transplanted gp33 expressing tumor in mice immunized by i.ln. injection of gp33 epitope-expressing, or control, plasmid.

Figure 10. Amount of plasmid DNA detected by real-time PCR in injected or draining lymph nodes at various times after i.ln. of i.m. injection, respectively.

Figure 11. Average % supercoiled DNA in formulations 1-9 over 7 days.

DETAILED DESCRIPTION OF THE INVENTION

Method of treatment

One aspect of this invention is a method for inducing or sustaining a specific immunological response (i.e., a CTL response) in an animal that has a disease (or predisposition to a disease) in which the animal's immune system may attack the disease with a natural CTL response. The response and diseases are discussed in greater detail hereinafter. The method has particular value for treating an animal having a malignant tumor in order to inhibit the growth of the tumor or for treating a chronic infectious disease such as hepatitis or AIDS.

The method, along with other aspects of the invention, is useful in an animal having an immune system that includes a lymphatic system. This generally includes vertebrates, specifically mammals and particularly humans. Thus, this invention will find use in treating humans of all ages as well as in treating animals, i.e. in veterinary uses. The invention may be used for treating livestock such as cattle, sheep, pigs, goats, and the like or for treating household pets such as dogs, cats, rabbits, hamsters, mice,

rats, and the like. The primary use will be for treating humans that are in need of having a specific immunological response sustained for treatment of a disease such as cancer or chronic infections.

A key aspect of this invention is the delivery of an appropriate antigen to the lymphatic system of the animal being treated and sustaining the delivery over time. This is based in part on the observation that a strong induction and a sustained CTL response require ongoing antigenic stimulation of the lymphatic system. In a human, the lymphatic system includes lymph, lymphocytes, lymph vessels, lymph nodes, tonsils, the spleen, the thymus gland, and bone marrow. The lymphatic system performs three basic functions. First, it helps maintain fluid balance in the tissues. Approximately 30 L of fluid pass from the blood capillaries into the interstitial spaces each day, whereas only 27 L pass from the interstitial spaces back into the blood capillaries. If the extra 3 L of interstitial fluid were to remain in the interstitial spaces, edema would result, causing tissue damage and eventual death. These 3 L of fluid (i.e. lymph) enter the lymph capillaries, then passes through the lymph vessels to return to the blood. Lymph is similar in composition to plasma. In addition to water, lymph contains solutes derived from two sources: (1) substances in plasma such as ions, nutrients, gases, and some proteins pass from blood capillaries into the interstitial spaces to become part of the lymph; and (2) substances derived from cells within the tissues such as hormones, enzymes, and waste products are also found in lymph.

The lymphatic system's second basic function is to absorb fats and other substances from the digestive tract. Special lymph vessels called lacteals are in the lining of the small intestine. Fats enter into the lacteals and pass through the lymph vessels to the venous circulation. The lymph passing through these capillaries has a milky appearance because of its fat content, and it is called chyle.

The third basic function of the lymphatic system is to act as part of the body's defense system. The lymph nodes filter lymph, and the spleen filters blood, removing microorganisms and other foreign substances. This third function is the function most important to this invention in that the antigen must be delivered to the lymph system at a level sufficient to elicit the desired, specific immunological response in the animal. Figure 5 is a schematic representation of a human lymphatic system showing the major lymphatic organs and vessels.

As hereinbefore mentioned, the present invention relates to a method of inducing or sustaining a specific immunological response (particularly a CTL response) to an antigen in an animal over time. The method comprises delivering the antigen to the animal in a manner that delivers the antigen into the lymphatic system of an animal to sustain the desired response over time. Generally this is done by establishing a mechanism to transfer an antigen from a reservoir to the animal's lymphatic system on a regular basis over time. The antigen may be delivered by a variety of methods that target intralymphatic presentation, including subcutaneous injection, direct injection into the lymphatic system by an antigen delivery vehicle that is implanted, preferably at or near a lymphatic organ, or by an antigen delivery vehicle that is external to the animal but contains a means (e.g. a needle or catheter) to deliver the antigen into the lymphatic system. By this method one can avoid multiple ongoing injections and can also avoid the use of including professional antigen-presenting cells in the composition held in the reservoir.

The method of this invention can be viewed as inducing CTL immune response by providing high continuous local concentrations of antigen, which otherwise is quickly removed and degraded from the body after bolus injection. Potent activation of CD8+T cells requires signaling through the T cell receptor (TCR) in a manner that is dependent on both quantitative and qualitative factors. Quantitative factors refer to the number of TCRs engaged by peptide-MHC complexes. Qualitative considerations include the duration of engagement of the TCR by peptide-MHC complexes, with specific peptide-MFIC complexes. Sustained regular deliveries of antigen allows optimal conditions to be established for inducing CD8+ T cells.

The antigen is delivered to the animal so that the antigen is present in the animal's lymphatic system on a sustained basis over a period of time. That is to say, it is delivered in such a way that the presence of the antigen is maintained over the period of time in the animal's lymphatic system. Thus, the antigen is delivered to the animal on a regular basis, i.e. the antigen is delivered regularly without significant interruption over the period of time. This regular delivery is achieved by the constant delivery of the antigen at low levels directly to the lymphatic system using an external device or an implantable device, as discussed hereinafter. Alternatively, the antigen can be delivered at higher levels to the animal by subcutaneous injection with indirect absorption or

equilibration with the lymph system. Delivery on a regular basis is meant to include intermittent (stopping and transmitting at intervals) as well as continuous (transmitting without interruption) delivery. In intermittent delivery, the times transmission is stopped will not be enough to reduce the level of antigen in the animal's lymphatic system to eliminate the desired specific immunological response. Thus, the antigen may be delivered in pulses or small doses over time.

Preferably, the sustained delivery is achieved by the positioning of a means of delivery so that the animal being treated does not have to receive multiple injections of the antigen, but instead has only one insertion of the means for delivery, e.g. an insertion of a catheter or needle for infusion of a suitable antigen-containing composition or the surgical implantation of an implantable device that releases an appropriate, antigen-containing composition on a sustained basis.

The period of time over which the antigen will be released will be a time sufficient to induce and maintain the desired specific immunological response, e.g. to maintain a CTL response, and in the case of an animal with a tumor or infection, at a level sufficient to stimulate the immune system to attack the tumor and inhibit its growth or to attack the infection. Generally, this period of time may vary from a few days, e.g. a week, to a year or more. Preferably, the treatment, i.e. sustained delivery of the antigen, will extend for at least seven days and no more than six months. It has been found that the CTL response is induced by administration for at least seven days. To determine the period of time, the attending physician will evaluate, i.e., the severity of the condition, the strength of the patient, the antigenic response (e.g., the level of CD8+ cells measurable in the patient's system), the presence of toxic effects, and other factors known to one of skill in the art. Ultimately the time for sustained delivery in a cancer patient will be that necessary for improvement in the patient as evidenced by reduction in the size of the tumor, the rate of growth of the tumor, and/or the improvement in the overall health of the patient being treated. In the treatment of infectious diseases the treatment is continued until the health of the patient improves sufficiently to stop treatment.

The underlying immunological rationale for the utility of this invention arises from certain immunological considerations, The immune system has evolved to protect the host from microbial infection. CD4+ T cells together with B cells are the main

components of the immune system humoral effector arm, which is crucial to eliminate extracellular pathogens or toxins. In contrast, the CD8+ T cell arm of the immune system is mainly responsible for eliminating intracellular pathogens, i.e. most importantly viruses, either via cytokine release or by cytotoxic activity. It is now emerging that these most efficient "killer cells" of the immune system would best serve as the primary effector cells in tumor immunotherapy. An object of this invention is to mount a disease-specific CTL response (CD8+ T cell response) against the disease and sustain it over time, e.g., a tumor specific or microbial specific CTL response.

CD8+ T cells recognize antigenic oligopeptides presented on HLA class I molecules of target cells, e.g., tumor cells. The sequences of many HLA-Al and HLA-A2 presented tumor and pathogen specific antigen peptides have recently been characterized. These peptides may be used in this invention to induce, e.g., a melanoma-specific CD8+ T cell response. These peptides are discussed hereinafter.

In contrast to viral infection, class I-binding oligopeptides show only low immunogenicity. Most viruses induce peak CD8+ T cell responses around 7-10 days after systemic spread. This invention aims at enhancing the immunogenecity of class I binding oligopeptides by sustained, regular release of peptide into a lymphatic system .and continued release into the lymphatic system.

In contrast to antibody-mediated B cell memory, which is long lived, T cell memory appears to be short lived or non-existent. In accordance with this invention, maintenance of functional T cell memory depends on persistence of antigen through continued, regular administration of the desired antigen. Having made this invention and looking at past concepts that might support this underlying rationale, some evidence includes the observation that delayed type hypersensitivity (DTH) of the tuberculin type (the only functional test for T cell memory in humans), can be elicited only in granulomatous disease, such as tuberculosis (tuberculin test), leprosy (lepromin test), brucellosis (brucellin test), sarcoidosis (Kveim test), Histoplasmosis (histoplasimin test) etc., but no such test could be established for non-granulomatous infectious disease. A factor that all granulomatous diseases have in common, is that the antigen persists within the granuloma – professional antigen presenting cells can use this reservoir to continuously restimulate specific T cells in lymphoid organs. In mice models (see

Example 3) it is demonstrated that maintenance of functional CD8+T cell memory was strictly dependent on continuous antigenic restimulation.

To determine whether a CTL response is obtained in an animal being treated in accordance with this invention, one measures the level of CD8+ cells (i.e. CTL) present in the blood or lymphatic organs such as the spleen or lymph nodes. This determination is done by first measuring the level of CD8+ cells before performing the method of this invention and measuring the level during treatment, e.g. at 7, 10, 20, 40 days, etc. The level or strength of the CD8+ (CTL) response can be assessed in vivo or in vitro. In humans, there exists so far only one in vivo test to measure CD8+ T cell responses, which is a skin test. In this skin test, HLA class I binding peptides are injected, intradermally (such as described in Jäger, E. et al. Granulocyte-macrophage-colony-stimulating Factor Enhances Immune Responses To Melanoma-associated Peptides in vivo Int. J Cancer 67, 54-62 (1996)). If a CTL response is present, these cells will recognize and attack peptide pulsed dermal cells, causing a local inflammatory reaction either via cytokine release or the cytotoxic mechanism (Kündig, T.M., Althage, A., Hengartner, H. & Zinkernagel, R.M. A skin test to assess CD8+ cytotoxic T cell activity. Proc. Natl. Acad Sci. USA 89:7757-776 (1992)). This inflammatory reaction can be quantified by measuring the diameter of the local skin rash and/or by measuring the diameter of the infiltrate (i.e., the swelling reaction). As an alternative to the injection of soluble free peptide, the HLA-class I binding peptide can also be injected intradermally in a bound form, e.g., bound to extracorporally derived dendritic cells. In other mammals, additional, although experimental, in vivo tests to assess CD8+ T cell responses exist. For example, in a mouse model, CD8+ T cell responses can be measured by challenge infection with a vaccinia recombinant virus expressing the peptide used for immunization. While naïve mice succumb to the infection with the vaccina recombinant virus, mice with preexisting CD8+ T cell immunity against the peptide epitope expressed by the vaccinia recombinant virus, are immune to reinfection. The level of immunity to reinfection can be quantified as the factor of reduction of the vaccinia virus titer recovered from mouse organs after challenge infection (Bachmann, M.F. & Kundig, T.M. In vitro vs. in vivo assays for the assessment of T-and B- cell function. Curr. Opin. Immunol. 6, 320-326 (1994)). For example, 5 days after challenge infection, a typical vaccinia recombinant virus titer recovered from a mouse ovary

would be around 10^7 pfu per ovary, whereas the vaccinia recombinant virus titer in a mouse with a preexisting CD8+ T cell response against the recombinant gene product would for example be around 10^3 pfu per ovary. Such a 10,000 fold-reduction in virus titer reflects biologically significant preexisting CD8+ T cell activity against the recombinant gene product.

The level of CD8+ T cell responses can also be quantified in vitro, by estimating the number of CD8+ T cells specific for the antigenic peptide in question. In a naïve mammal the so called "frequency", i.e., the number of specific CD8+ T cells divided by the number of non-specific white blood cells, is less than 10^{-6}. After successful immunization, the frequency increases due to proliferation of specific T cells. During an acute viral infection, for example, the frequency of specific CD8+ T cells may rise to 10^{-2} . Then, after elimination of the virus, the frequency of specific CD8+ T cells usually drops to a "memory" level of around 10^{-4}. Thus, the CD8+ T cell response can be quantified by measuring the frequency of specific CD8+ T cells. The higher the frequency, the stronger the response. The classical assays used to measure the frequency of specific CD8+ T cells are based on limiting dilution cell culture techniques, as described in detail by Kündig, T.M. et al. (On the role of antigen in maintaining cytotoxic T cell memory. Proceedings of the National Academy of Sciences of the United States of America 93, 9716-972') (1996)). A novel approach to estimate the frequency of specific CD8+ T cells is to construct soluble class I MHC (for use in mice) or HLA molecules (for use in humans) with a peptide bound to their groove, so that the specific T cell receptors will bind to these complexes. These complexes can be labeled for detection, for example, with a fluorescent substance, allowing for detection by flow cytometry.

One current procedure to render peptides immunogenic is to inject them in context with "nature's most potent adjuvant", i.e., professional antigen presenting cells (APCs) such as dendritic cells (DCs), (Steinmann, R.M., The dendritic cells system and its role in irnmunogenicity, Annual Review of Immunology 9, 271-96 (1991)). DCs are the most potent APCs of the immune system. They can now be cultured in vitro by adding granulocyte macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-alpha) or interleukin-4 (IL-4) to progenitors isolated from the blood of patients or mice (Inaba, K. el al., Identification of proliferating dendritic

cell precursors in mouse blood, Journal of Experimental Medicine 175, 1157-1167 (1992)). Large numbers of DCs can then be pulsed with tumor specific antigen peptides and are injected back into the patient, where they migrate into lymphatic organs to induce T cell responses (Young, J.W. & Inaba, K., Dendritic Cells As Adjuvants For Class I Major Histocompatibility Complex-restricted Anti-tumor Immunity, Journal of Experimental Medicine 183, 7-11 (1996)). An object of this invention is to circumvent the time-consuming, labor intensive procedure of culturing DCs after isolation of DC progenitors and deliver the antigen to the lymphatic system free of APCs such as DCs. The method of this invention, i.e., the sustained, regular delivery of antigen into a lymphatic organ, allows sufficiently high local concentrations of antigen inside the lymphatic organ, such that professional antigen presenting cells, e.g., dendritic cells, can be loaded with peptide in vivo. This can be viewed as a method of loading antigen presenting cells (dendritic cells) in vivo for inducing a CTL response.

The method of the present invention is clearly advantageous over the prior art methods for inducing a CTL response against a tumor or virus. For example, the present invention does not require repetitive immunizations to effect for prolonged anti-tumor immunotherapy. The sustained delivery of the antigen maintains the CTL response that could ultimately afford a prolonged aggressive posture of CTL against tumor cells, more thorough eradication, and protection against recurrence during the vaccine treatment. In the absence of antigen, CTL that have undergone primary activation soon cease to recirculate through the body, soon finding their way to the spleen where they become quiescent. Since CTL must immediately deliver a lethal hit, their residence in the spleen precludes an active role in protection against infections or tumor growth at distant sites in the body. The controlled release of antigen recognized by CTL in this invention circumvents this outcome as antigen delivery is maintained. Sustained released antigen delivery to the lymphatic system by this invention solves two major problems: it provides for potent CTL stimulation that takes place in the milieu of the lymphoid organ, and it sustains stimulation that is necessary to keep CTL active, cytotoxic and recirculating through the body.

Another fundamental improvement of the present method over prior art is that it facilitates the use of inherently non-immunogenic peptide antigens for CTL stimulation without the combined use of conventional adjuvants. This is very beneficial as most

experimental adjuvants are toxic and poorly suited for use in humans. In addition adjuvants stimulate the TH2-type humoral immune response that negatively affects the CTL response. Further, since conventional adjuvants are not required, only the minimal antigenic epitope for a CTL response is required in the formulation.

An additional advantage to the method of the present invention, where it embodies the use of mechanical delivery systems, is that the antigen delivery can be stopped if any adverse immunological effects are observed, For example, in vaccines against melanoma, CTL have been induced to attack not only malignant melanocytes but also healthy tissue, causing "vitiligo." The ability to discontinue a CTL vaccine at any time is a significant advance in vaccine safety. Peptides have a short half-life due to catabolism in the liver. Therefore, the stimulation-effect falls soon after cessation of delivery.

As pointed out before, the method of this invention has two parts: (1) inducing an increased CTL response and (2) maintaining the response. The inducing and maintaining may be performed using the same device, as discussed hereinafter, or the inducing may be done separately, e.g., by a separate injection of an antigen then following up with sustained delivery of the antigen over time to maintain the response.

Diseases treated according to the invention

In general, this invention is useful for treating an animal having (or being predisposed to) any disease to which the animal's immune system mounts a cell-mediated response to a disease-related antigen in order to attack the disease. Thus, the type of disease may be a malignant tumor or a chronic infectious disease caused by a bacterium, virus, protozoan, helminth, or other microbial pathogen that enters intracellularly and is attacked, i.e., by the cytotoxic T lymphocytes, In addition, the invention is useful for treating an animal that may be at risk of developing such diseases.

Malignant Tumors

In a mature animal, a balance usually is maintained between cell renewal and cell death in most organs and tissues. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of stem cells. Under normal circumstances, the production of new cells is so regulated that the numbers of any particular type of cell

remain constant. Occasionally, though, cells arise that are no longer responsive to normal growth-control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor, or neoplasm. A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is benign. A tumor that continues to grow and becomes progressively invasive is malignant; the term cancer refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit metastasis; in this process, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site. The methods, devices and articles of manufacture discussed herein are useful for treating animals having malignant tumors.

Malignant tumors treated according to this invention are classified according to the embryonic origin of the tissue from which the tumor is derived. Carcinomas are tumors arising from endodermal or ectodermal tissues such as skin or the epithelial lining of internal organs and glands. A melanoma is a type of carcinoma of the skin for which this invention is particularly useful. Sarcomas, which arise less frequently, are derived from mesodermal connective tissues such as bone, fat, and cartilage. The leukemias and lymphomas are malignant tumors of hematopoietic cells of the bone marrow. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. The malignant tumors may show up at numerous organs or tissues of the body to establish a cancer. The types of cancer that can be treated in accordance with this invention include the following: bladder, brain, breast, cervical, colo-rectal, esophageal, kidney, liver, lung, nasopharangeal, pancreatic, prostate, skin, stomach, uterine, and the like. The present invention is not limited to the treatment of -an existing tumor or infectious disease but can also be used to prevent or lower the risk of developing such diseases in an individual, ie., for prophylactic use. Potential candidates for prophylactic vaccination include individuals with a high risk of developing cancer, i.e., with a personal or tuminal history of certain types of cancer.

The incidence of skin cancer has increased substantially over the last decades. Lifetime analysis indicates that around 1/1500 humans born in 1935, 1/600 born in 1960, 1/100 born in 1990 and a projected 1/75 humans born in the year 2000 will have

melanoma in their lifetime. Surgical excision usually cures melanoma. However, even small looking lesions may have already metastasized at the time of diagnosis. The prognosis of metastasized melanoma is very poor and correlates with the thickness of the primary tumor and with its localization.

The current treatment of malignant melanoma aims at surgical removal of the primary tumor, If metastases are present, chemotherapy and biological response modifiers are additionally used. However, patients with stage IV malignant melanoma are almost invariably incurable and treatments are palliative. Patients with Stage IV malignant melanoma have a median survival time of approximately one year and only a 10% chance of long-term survival. There is at present no generally accepted standard therapy for metastatic melanoma. Objective response rates to mono- or polychemotherapy are low in comparison with other tumors, reaching no more than 15-35%. An improved treatment outcome in stage IV malignant melanoma seems unachievable either by chemotherapeutic combinations or by increasing doses to levels where autologous bone marrow transplantation becomes necessary. The method of this invention is useful for treating malignant melanoma, even at Stage IV.

Infectious Diseases

Infectious diseases, which have plagued animal populations (particularly humans) throughout- history, still cause millions of deaths each year. The infectious diseases that can be treated using this invention include those caused by pathogens such as bacteria, viruses, protozoa, helminths, and the like. These diseases include such chronic diseases such as acute respiratory infections, diarrheal diseases, tuberculosis, malaria, hepatitis (hepatitis A, B C, D, E, F virus), measles, mononucleosis (Epstein-Barr virus), whooping cough (pertussis), AIDS (human immunodeficiency virus I & 2), rabies, yellow fever, and the like. Other diseases caused by human papilloma virus or various strains of virus are treatable by this method.

In some instances, the mammal, in particular human, can be treated prophylactically, such as when there may be a risk of developing disease. An individual travelling to or living in an area of endemic infectious disease may be considered to be at risk and a candidate for prophylactic vaccination against the particular infectious agent. For example, the CTL response can be induced in a human expecting to enter a malarial area and/or while in the malarial area by using a CTL-inducing,

-malaria-specific antigen to lower the risk of developing malaria. Preventative treatment can be applied to any number of diseases including those listed above, where there is a known relationship between the particular disease and a particular risk factor, such as geographical location or work environment.

Antigens useful in the invention

An antigen useful in this invention is one that stimulates the immune system of a mammal having a malignant tumor or infectious disease to attack the tumor and inhibit its growth or to destroy the pathogen causing the disease. Thus, the antigen used in the invention is matched to the specific disease found in the animal being treated. In this regard the antigen may be said to induce a CTL response (also referred to as a cell-mediated immune response), i.e. a cytotoxic reaction by the immune system that results in lysis of the target cells (e.g., the malignant tumor cells or pathogen-infected cells).

To determine whether an antigen is matched to a particular patient, whether human or other animal, the tissue type of the patient is first determined. If human, the tissue must demonstrate the appropriate human leukocyte antigen (HLA) capable of binding and displaying the antigen to CTL. It is preferable that the HLA typing be performed, on the target cells, since a significant portion of tumors escape immune detection by downregulating expression of HLA. Therefore HLA expression on normal cells of the patient does not necessarily reflect that found on tumor cells in their body. A tumor from a patient is also screened to determine if he or she expresses the antigen that is being used in the vaccine formulation. Immunohistochemistry and/or polymerase chain reaction (PCR) techniques both can be used to detect antigen in the tumor cells. Immunchistochemistry offers the advantage in that it stains a cross-section of tumor in a slide preparation, allowing investigators to observe the antigen expression pattern in cross-section of tumor, which is typically heterogeneous for antigen expression. PCR has the advantage of not requiring specific monoclonal antibodies for staining and is a fast and powerful technique. In addition, PCR can be applied in situ. Ideally, both immunohistochemical and PCR methods should be combined when assessing antigen expression in tumors. While the antigen compositions useful in this invention are designed to include the most commonly expressed tumor antigens (as discussed hereafter), not all tumors will express the desired antigen(s). Where a tumor fails to

express the desired antigen, the patient is excluded for consideration for that particular antigen composition. Thus, an aspect of this invention is a process for preparing a device useful for providing a sustained CTL response over time by matching a subject's antigen specific to the tumor or pathogen in the subject, preparing a physiologically-acceptable composition of the antigen so matched, and combining the composition in a suitable delivery device as discussed in hereinafter.

Immune activation of CD8+ T cells generates a population of effector cells with lytic capability called cytotoxic T lymphocytes, or CTL. These effector cells have important roles in the recognition and elimination of malignant cells and pathogens. In general, CTL are CD8+ and are therefore class I MHC restricted, although in rare instances CD4+ class II - restricted T cells have been shown to function as CTL. Since virtually all nucleated cells in the body express class I MHC molecules, CTL can recognize and eliminate almost any altered body cell. CD8+T cells recognize antigen presented on HLA class I molecules of tumor cells through T cell receptors.

The CTL-mediated immune response can be divided into two phases, reflecting different aspects of the cytotoxic T-cell response. The first phase involves the activation and differentiation of T_c (CD8+) cells into functional effector CTLs. In the second phase, CTLs, recognize antigen - class I MHC complexes on specific target cells, initiating a sequence of events that culminates in target-cell destruction. Further detailed discussion of the process is found at Chapter 15 of the Second Edition of "Immunology" by Janis Kuby, W.H. Freeman and Company (199 1).

The type of tumor antigen used in this invention may be a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA), A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA associated antigen is not unique to a tumor cell and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development when the immune system is immature and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells but which are expressed at much higher levels on tumor cells. TSAs and TAAs can be jointly referred to as TRA or a tumor related antigen.

Tumor antigens useful in the present invention, whether tumor-specific or tumor-associated, must be capable of inducing a CTL-mediated immune response. The presence of tumor antigens that elicit a cell-mediated response has been demonstrated by the rejection of tumors transplanted into syngeneic recipients; because of this phenomenon, these tumor antigens are referred to as tumor-specific transplantation antigens (TSTAs) or tumor-associated transplantation antigens (TATAs). It has been difficult to characterize tumor transplantation antigens because they do not generally elicit an antibody response and therefore they cannot be isolated by immunoprecipitation. Many are peptides that are presented together with MHC molecules on the surface of tumor cells and have been characterized by their ability to induce an antigen-specific CTL.

The type of pathogen specific antigen useful in this invention may be short oligopeptides derived from pathogen proteins. These oligopeptides must bind to class I MHC (for use in mice), class I HLA (for use in humans), or class I molecules of any other mammals. Also, such class I molecule bound peptides should be recognizable by specific T cell receptors. Such oligopeptides usually have a length of 8-15 amino acids. Several examples of such pathogen derived oligopeptides, so called T cell epitopes, are given in Tables I and II.

The tumor antigens and pathogen-specific antigens useful in this invention are generally thought to be presented at the surface of an antigen presenting cell (APC) to stimulate the immune system through class I molecules of the major histocompatability complex (MHC) interactively with the CD8+ cells.

Antigens useful in the invention are generally protein-based entities of a molecular weight of up to 100,000 daltons. Appropriate antigens include, but are not limited to differentiation antigens, tumor-specific multilineage antigens, embryonic antigens, antigens of oncogenes and mutated tumor-suppressor genes, unique tumor antigens resulting from chromosomal translocations, viral antigens, and others that may be apparent presently or in the future to one of skill in the art. It is preferable that the antigen be a peptide of 8 to 15 amino acids in length that is an epitope of a larger antigen, i.e. it is a peptide having an amino acid sequence corresponding to the site on the larger molecule that is recognized and bound by a particular T-cell receptor. These smaller peptides are available to one of skill in the art by following the teachings of

U.S. Patents 5,747,269 to Rammensee et al. issued May 5, 1998; 5,698,396 to Pfreundschuh issued December 16, 1997; and PCT Application Numbers PCT/EP95/02593 filed 4 July 1995, PCT/DE96/00351 filed 26 Feb 1996, all of which are incorporated herein by reference. Additional approaches to epitope discovery are described in U.S. Patent 6,037,135 METHODS FOR MAKING HLA BINDING PEPTIDES AND THEIR USES and U.S. Patent Application No. 09/561,074 entitled METHOD OF EPITOPE DISCOVERY both of which are incorporated herein by reference in their entirety.

While in the general case the antigen ultimately recognized by a T cell is a peptide, it must be kept in mind that the form of antigen actually administered as the immunogenic preparation need not be a peptide *per se*. When administered, the epitopic peptide(s) may reside within a longer polypeptide, whether the complete protein antigen, some segment of it, or some engineered sequence. Included in such engineered sequences would be polyepitopes and epitopes incorporated into some carrier sequence such as an antibody or viral capsid protein. Such longer polypeptides may include epitope clusters as described in U.S. Patent Application No. 09/561,571 entitled "EPITOPE CLUSTERS," which is incorporated herein by reference in its entirety. The epitopic peptide, or the longer polypeptide in which it is contained, may be a component of a microorganism (e.g. a virus, bacterium, protozoan, etc.), or a mammalian cell (e.g. a tumor cell or antigen presenting cell), or lysates, whole or partially purified, of any of the foregoing. They may be used as complexes with other proteins, for example heat shock proteins. The epitopic peptide may also be covalently modified, such as by lipidation, or made a component of a synthetic compound, such as dendrimers, multiple antigen peptides systems (MAPS), and polyoximes, or may be incorporated into liposomes or microshperes, etc. As used in this disclosure the term "polypeptide antigen" encompasses all such possibilities and combinations. The invention comprehends that the antigen may be a native component of the microorganism or mammalian cell. The antigen may also be expressed by the microorganism or mammalian cell through recombinant DNA technology or, especially in the case of antigen presenting cells, by pulsing the cell with polypeptide antigen prior to administration. Additionally, the antigen may be administered encoded by a nucleic acid that is subsequently expressed by APCs. Finally, whereas the classical class I MHC

molecules present peptide antigens, there are additional class I molecules which are adapted to present non-peptide macromolecules, particularly components of microbial cell walls, including without limitation lipids and glycolipids. As used in this disclosure the term antigen comprehends such macromolecules as well. Moreover, a nucleic acid based vaccine may encode an enzyme or enzymes necessary to the synthesis of such a macromolecule and thereby confer antigen expression on an APC.

A powerful method has been recently developed for identifying new peptides that are useful in the invention. Genes determined to express protein with high exclusivity in tumor cells or microbial cells (e.g. viruses) can be identified using a so called SEREX process, which involves expression cloning using tumor cell libraries and screening these libraries against immunoglobulin in patient sera. Over one hundred genes have recently been identified from tumor biopsies using this process. These genes can now be used in a peptide prediction algorithm developed by Hans-Georg Rammensee. Algorithms have been developed for all major HLA types found in the human population. First the protein sequence is "translated" based on the gene sequence. The algorithms can predict peptide epitopes for various HLA types based on the protein sequence. Since the predicted peptides are indeed predictions and are not always naturally found on cells, tumor samples are used to confirm the predicted peptides by actually isolating minute trace peptide from tumors. Being able to calculate the exact mass of the predicted peptides allows trace peptide identification using ultrasensitive mass spectrophotometry, which can detect peptides in quantities less that that which would permit peptide sequencing and identification. Once these tumor-associated peptides have been identified they are suitable for use in the invention, since peptides of a known sequence may be synthesized in large quantities (several grams) providing for sufficient amounts of peptides for use in this invention.

In addition to the imperfection of existing prediction algorithms for MHC binding, some peptides that would be fully capable of binding to MHC may never be liberated by protoelytic processing from the complete protein antigen. Methods for evaluating which peptides will be liberated by proteasomal processing have been developed, e.g. U.S. Patent Application No. 09/561,074 *supra*, increasing the efficiency with which useful epitopes can be discovered. Moreover, proteasomal processing can differ between target cell and APC such that care must be taken in the identification and

selection of epitopes and in vaccine design so that the vaccine will induce a response that will in fact recognize the target cell. These issues are more fully discussed in U.S. Patent Application No. 09/560,465 entitled "EPITOPE SYNCHRONIZATION," which is incorporated herein by reference in its entirety.

Thus it can be seen that another aspect of this invention is a process for preparing a composition useful in a device of this invention as discussed hereinafter. The process comprises identifying a gene determined to express a protein with high exclusivity in a tumor or microbial cell, cloning cell libraries, screening the libraries against immunoglobulin in patient sera, using the algorithm defined in the literature developed by Hans-George Rammensee to predict an epitope for the HLA type protein based on the gene sequence, matching the predicted antigen sequence to a patient tumor sample, isolating the matched antigen, and preparing a composition of the antigen for use in a delivery device as discussed hereinafter.

Examples of large, protein-based antigens include the following:

Differentiation antigens such as MART-1/MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER-2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, p185erbB2, p180erbB-3, c-met, nm-23HI, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum- 1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein , ß-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS. These protein-based antigens are known and available to those of skill in the art in the literature or commercially.

Examples of peptide antigens of 8-15 amino acids include those set forth 'in Table I, Table II, and Table III. Table I sets forth antigens that are virally derived. The

Table shows the virus type, the protein expressed by the virus, the amino acid (AA) position on the viral protein, the AA sequence of the T-cell epitope/MHC ligand, the type of MHC molecule presenting the antigen, and a reference source. A more complete list is provided in the book by Han-Georg Rammensee, Jutta Bachmann, and Stefan Stevanovic entitled "MHC Ligands and Peptide Motifs," Springer-Verlag, Germany, 1997 Landes Bioscience, Austin, Texas). The reference number given in Table I is the same number (and reference source) given in Table 5.3 of the above Rammensee book, all of which is incorporated herein by reference.

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
Adenovirus 3	E3 9Kd	30-38	LIVIGILIL (SEQ. ID NO.: 1)	HLA-A*0201	104
Adenovirus 5	EIA	234-243	SGPSNTPPEI (SEQ. ID NO.:2)	H2-Db	105
Adenovirus 5	EIB	192-200	VNIRNCCYI (SEQ. ID NO.:3)	H2-Db	106
Adenovirus 5	EIA	234-243	SGPSNIPPEI (T>I) (SEQ. ID NO.:4)	H2-Db	106
CSFV	NS polyprotein	2276-2284	ENALLVALF (SEQ. ID NO.:5	SLA,haplotype d/d	107
Dengue virus 4	NS3	500-508	TPEGIIPTL (SEQ. ID NO.:6	HLA-B*3501	108,109
EBV	LMP-2	426-434	CLGGLLTMV (SEQ. ID NO.:7)	HLA-A*0201	110
EBV	EBNA-1	480-484	NIAEGLRAL (SEQ. ID NO.:8)	HLA-A*0201	111
EBV	EBNA-1	519-527	NLRRGTALA (SEQ. ID NO.:9)	HLA-A*0201	111
EBV	EBNA-1	525-533	ALAIPQCRL (SEQ. ID NO.: 10)	HLA-A*0201	111
EBV	EBNA-1	575-582	VLKDAIKDL (SEQ. ID NO.: 11)	HLA-A*0201	111
EBV	EBNA-1	562-570	FMVFLQTHI (SEQ. ID NO.: 12)	HLA-A*0201	111
EBV	EBNA-2	15-23	HLIVDTDSL (SEQ. ID NO.:13)	HLA-A*0201	111
EBV	EBNA-2	22-30	SLGNPSLSV (SEQ. ID NO.: 14)	HLA-A*0201	111
EBV	EBNA-2	126-134	PLASAMRML (SEQ. ID NO.: 15)	HLA-A*0201	111
EBV	EBNA-2	132-140	RMLWMANYI (SEQ. ID NO.: 16)	HLA-A*0201	111
EBV	EBNA-2	133-141	MLWMANYIV (SEQ. ID NO.: 17)	HL.A-A*0201	111
EBV	EBNA-2	151-159	ILPQGPQTA (SEQ. ID NO.: 18)	HLA-A*0201	111
EBV	EBNA-2	171-179	PLRPTAPTI (SEQ. ID NO.: 19)	HLA-A*0201	111
EBV	EBNA-2	205-213	PLPPATLTV (SEQ. ID NO.:20)	HLA-A*0201	111
EBV	EBNA-2	246-254	RMHLPVLHV (SEQ, ID NO.:21)	HLA-A*0201	111
EBV	EBNA-2	287-295	PMPLPPSQL (SEQ, ID NO.:22)	HLA-A*0201	111
EBV	EBNA-2	294-302	QLPPPAAPA (SEQ, ID NO.:23)	HLA-A*0201	111
EBV	EBNA-2	381-389	SMPELSPVL (SEQ. ID NO.:24)	HLA-A*0201	111
EBV	EBNA-2	453-461	DLDESWDYI (SEQ. ID NO.:25)	HLA-A*0201	111
EBV	BZLFl	43-51	PLPCVLWPV (SEQ. ID NO.:26)	HLA-A*0201	111

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
EBV	BZLF1	167-175	SLEECDSEL (SEQ. ID NO..27)	HLA-A*0201	111
EBV	BZLF1	176-184	EIKRYKNRV (SEQ. ID NO.:28)	HLA-A*0201	111
EBV	BZLF1	195-203	QLLQHYREV (SEQ. ID NO.:29)	HLA-A*0201	111
EBV	BZLF1	196-204	LLQHYREVA (SEQ. ID NO.:30)	HLA-A*0201	111
EBV	BZLFI	217-225	LLKQMCPSL (SEQ. ID NO.:31)	HLA-A*0201	111
EBV	BZLF1	229-237	SIIPRTPDV (SEQ. ID NO.:32)	HLA-A*0201	111
EBV	EBNA-6	284-293	LLDFVRFMGV (SEQ. ID NO.:33)	HLA-A*0201	112
EBV	EBNA-3	464-472	SVRDRLARL (SEQ. ID NO.:34)	HLA-A*0203	113
EBV	EBNA-4	416-424	IVTDFSVIK (SEQ. ID NO.:35)	HLA-A*1101	114,115
EBV	EBNA-4	399-408	AVFDRKSDAK (SEQ. ID NO.:36)	HLA-A*0201	116
EBV	EBNA-3	246-253	RYSIFFDY (SEQ. ID NO.:37)	HLA-A24	113
EBV	EBNA-6	881-889	QPRAPIRPI (SEQ. ID NO.:38)	HLA-B7	117
EBV	EBNA-3	379-387	RPPIFIRRI. (SEQ. ID NO.:39)	HLA-B7	117
EBV	EBNA-1	426-434	EPDVPPGAI (SEQ. ID NO.:40)	HLA-B7	111
EBV	EBNA-1	228-236	IPQCRLTPL (SEQ. ID NO.:41)	HLA-B7	111
EBV	EBNA-1	546-554	GPGPQPGPL (SEQ. ID NO.:42)	HLA-B7	111
EBV	EBNA-1	550-558	QPGPLRESI (SEQ. ID NO.:43)	HLA-B7	111
EBV	EBNA-1	72-80	R.PQKRPSCI (SEQ. ID NO.:44)	HLA-B7	111
EBV	EBNA-2	224-232	PPTPLLTVL (SEQ. ID NO.:45)	HLA-B7	111
EBV	EBNA-2	241-249	TPSPPRMHL (SEQ. ID NO.:46)	HLA-B7	111
EBV	EBNA-2	244-252	PPRMHLPVL (SEQ. ID NO.:47)	HLA-B7	111
EBV	EBNA-2	254-262	VPDQSMHPL (SEQ. ID NO.:48)	HLA-B7	111
EBV	EBNA-2	446-454	PPSIDPADL (SEQ. ID NO.:49)	HLA-B7	111
EBV	BZLFI	44-52	LPCVLWPVL (SEQ. ID NO.:50)	HLA-B7	111
EBV	BZLF1	222-231	CPSLDVDSII (SEQ. ID NO.:51)	HLA-B7	111
EBV	BZLFI	234-242	TPDVLHEDL (SEQ. ID NO.:52)	HLA-B7	111
EBV	EBNA-3	339-347	FLRGRAYGL	HLA-B8	118

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
EBV	EBNA-3	26-34	(SEQ. ID NO.:53) QAKWRLQTL	HLA-B8	113
EBV	EBNA-3	325-333	(SEQ. ID NO.:54) AYPLHEQHG	HLA-B8	116
EBV	EBNA-3	158-166	(SEQ. ID NO.:55) YlKSFVSDA	HLA-B8	116
EBV	LMP-2	236-244	(SEQ. ID NO.:56) RRRWRRLTV	HLA-B*2704	119
EBV	EBNA-6	258-266	(SEQ. ID NO.:57) RRIYDLIEL	HLA-B*2705	119
EBV	EBNA-3	458-466	(SEQ. ID NO.:58) YPLHEQHGM	HLA-B*3501	120
EBV	EBNA-3	458-466	(SEQ. ID NO.:59) YPLHEQHGM	HLA-B*3503	113
HCV	NS3	389-397	(SEQ. ID NO.:59) HSKKKCDEL	HLA-B8	145
HCV	env E	44-51	(SEQ. ID NO.:60) ASRCWVAM	HLA-B*3501	146
HCV	core protein	27-35	(SEQ. ID NO.:61) GQIVGGVYL	HLA-B*40012	147
HCV	NSI	77-85	(SEQ. ID NO.:62) PPLTDFDQGW	HLA-B*5301	145
HCV	core protein	18-27	(SEQ. ID NO.:63) LMGYIPLVGA	H2-Dd	138
HCV	core protein	16-25	(SEQ. ID NO.:64) ADLMGYIPLV	H2-Dd	148
HCV	NS5	409-424	(SEQ. ID NO.:65) MSYSWTGALVTPC AEE	H2-Dd	149
HCV	NS1	205-213	(SEQ. ID NO.:66) KHPDATYSR	Papa-A06	150
HCV-1	NS3	400-409	(SEQ. ID NO.:67) KLVALGINAV	HLA-A*0201	141
HCV-1	NS3	440-448	(SEQ. ID NO.:68) GDFDSVIDC	Patr-B16	151
HCV-1	env E	118-126	(SEQ. ID NO.:69) GNASRCWVA	Patr-BI6	151
HCV-1	NSI	159-167	(SEQ. ID NO.:70) TRPPLGNWF	Patr-B13	151
HCV-1	NS3	351-359	(SEQ. ID NO.:71) VPHPNIEEV	Patr-B13	151
HCV-1	NS3	438-446	(SEQ. ID NO.:72) YTGDFDSVI	Patr-B01	151
HCV-1	NS4	328-335	(SEQ. ID NO.:73) SWAIKWEY	Patr-Al 1	151
HCV-1	NSI	205-213	(SEQ. ID NO.:74) KHPDATYSR	Patr-A04	150
HCV-1	NS3	440-448	(SEQ. ID NO.:75) GDFDSVIDC	Patr-A04	150
HIV	gp4l	583-591	(SEQ. ID NO.:76) RYLKDQQLL	HLA_A24	152
HIV	gagp24	267-275	(SEQ. ID NO.:77) IVGLNKIVR (SEQ. ID NO.:78)	HLA-A*3302	153,154

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HIV	gagp24	262-270	EIYKRWIIL (SEQ. ID NO.:79)	HLA-B8	155,156
HIV	gagp24	261-269	GEIYKRWI1 (SEQ. ID NO.:80)	HLA-B8	155,156
HIV	gagp17	93-101	EIKDTKEAL (SEQ. ID NO.:81)	HLA-B8	155,157
HIV	gp41	586-593	YLKDQQLL (SEQ. ID NO.:82)	HLA-B8	158
HIV	gagp24	267-277	ILGLNKIVRMY (SEQ. ID NO.:83)	HLA-B* 1501	153
HIV	gp41	584-592	ERYLKDQQL (SEQ. ID NO.:84)	HLA-B14	158
HIV	nef	115-125	YHTQGYFPQWQ (SEQ. ID NO.:85)	HLA-B17	159
HIV	nef	117-128	TQGYFPQWQNYT (SEQ. ID NO.:86)	HLA-B17	159
HIV	gpl20	314-322	GRAFVTIGK (SEQ. ID NO.:87)	HLA-B*2705	160, 184
HIV	gagp24	263-271	KRWIILGLN (SEQ. ID NO.:88)	HLA-B*2702	161
HIV	nef	72-82	QVPLRPMTYK (SEQ. ID NO.:89)	HLA-B*3501	159
HIV	nef	117-125	TQGYFPQWQ (SEQ. ID NO.:90)	HLA-B*3701	159
HIV	gagp24	143-151	HQAISPRTI, (SEQ. ID NO.:91)	HLA-Cw*0301	162
HIV	gagp24	140-151	QMVHQAISPRTL (SEQ. ID NO.:92)	HLA-Cw*0301	162
HIV	gpl20	431-440	MYAPPIGGQI (SEQ. ID NO.:93)	H2-Kd	163
HIV	gpl60	318-327	RGPGRAFVTI (SEQ. ID NO.:94)	H2-Dd	164, 165
HIV	gp120	17-29	MPGRAFVTI (SEQ. ID NO.:95)	H2-Ld	166, 167
HIV-1	RT	476-484	ILKEPVHGV (SEQ. ID NO.:96)	HLA-A*0201	168, 169
HIV-1	nef	190-198	AFHHVAREL (SEQ. ID NO.:97)	HLA-A*0201	170
HIV-1	gpI60	120-128	KLTPLCVTL (SEQ, ID NO.:98)	HLA-A*0201	171
HIV-1	gp]60	814-823	SLLNATDIAV (SEQ, ID NO.:99)	HLA-A*0201	171
HIV-1	RT	179-187	VIYQYMDDL (SEQ. ID NO.: 100)	HLA-A*0201	172
HIV-1	gagp 17	77-85	SLYNTVATL (SEQ. ID NO.: 101)	HLA-A*0201	173
HIV-1	gp160	315-329	RGPGRAFVT1 (SEQ. ID NO.: 102)	HLA-A*0201	174
HIV-1	gp41	768-778	RLRDLLLIVTR (SEQ, ID NO.: 103)	HLA-A3	175,178
HIV-1	nef	73-82	QVPLRPMTYK (SEQ. ID NO.: 104)	HLA-A3	176
HIV-1	gp120	36-45	TVYYGVPVWK	HLA-A3	177

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HIV-1	gagp17	20-29	(SEQ. ID NO.: 105) RLRPGGKKK	HLA-A3	177
HIV-1	gp120	38-46	(SEQ. ID NO.: 106) VYYGVPVWK	HLA-A3	179
HIV-1	nef	74-82	(SEQ. ID NO.: 107) VPLRPMTYK	HLA-a*1101	114
HIV-1	gagp24	325-333	(SEQ. ID NO.: 108) AIFQSSMTK	HLA-A*1101	114
HIV-1	nef	73-82	(SEQ. ID NO.: 109) QVPLRPMTYK	HLA-A*1101	180
HIV-1	nef	83-94	(SEQ. ID NO.: 104) AAVDLSHFLKEK	HLA-A*1101	159
HIV-1	gagp24	349-359	(SEQ, ID NO.:110) ACQGVGGPGGHK	HLA-A*1101	181
HIV-1	gagp24	203-212	(SEQ. ID NO.: 111) ETINEEAAEW	HLA-A25	182
HIV-1	nef	128-137	(SEQ. ID NO.: 112) TPGPGVRYPL	HLA-B7	159
HIV-1	gagp 17	24-31	(SEQ. ID NO.: 113) GGKKKYKL	HLA-B8	183
HIV-1	gpl20	2-10	(SEQ. ID NO.: 114) RVKEKYQHL	HLA-B8	181
HIV-1	gagp24	298-306	(SEQ. ID NO.: 115) DRFYKTLRA	HLA-B 14	173
HIV-1	NEF	132-147	(SEQ. ID NO.: 116) GVRYPLTFGWCYKL VP	HLA-B18	159
HIV-1	gagp24	265-24	(SEQ. ID NO.: 117) KRWIILGLNK	HLA-B*2705	184,153
HIV-1	nef	190-198	(SEQ. ID NO.: 118) AFHHVAREL	HLA-B*5201	170
EBV	EBNA-6	335-343	(SEQ. ID NO.:97) KEHVIQNAF	HLA-B44	121
EBV	EBNA-6	130-139	(SEQ. ID NO.: 119) EENLLDFVRF	HLA-B*4403	122
EBV	EBNA-2	42-51	(SEQ. ID NO.: 120) DTPLIPLTIF	HLA-B51	121
EBV	EBNA-6	213-222	(SEQ. ID NO.: 121) QNGALAINTF	HLA-1362	112
EBV	EBNA-3	603-611	(SEQ. ID NO.: 122) RLRAEAGVK	HLA-A3	123
HBV	sAg	348-357	(SEQ. ID NO.: 123) GLSPTVWLSV	HLA-A*0201	124
HBV	SAg	335-343	(SEQ. ID NO.: 124) WLSLLVPFV	HLA-A*0201	124
HBV	cAg	18-27	(SEQ. ID NO.: 125) FLPSDFFPSV	HLA-A*0201	125,126,127
HBV	cAg	18-27	(SEQ. ID NO.: 126) FLPSDFFPSV	HLA-A*0202	127
HBV	cAg	18-27	(SEQ. ID NO.: 126) FLPSDFFPSV	HLA-A*0205	127
HBV	cAg	18-27	(SEQ. ID NO.: 126) FLPSDFFPSV	HLA-A*0206	127
			(SEQ. ID NO.: 126)		

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HBV	pol	575-583	FLLSLGIHL (SEQ. ID NO.: 127)	HLA-A*0201	128
HBV	pol	816-824	SLYADSPSV (SEQ. ID NO.: 128)	HLA-A*0201	128
HBV	pol	455-463	GLSRYVARL (SEQ. ID NO.: 129)	HLA-A*0201	128
HBV	env	338-347	LLVPFVQWFV (SEQ. ID NO.: 130)	HLA-A*0201	129
HBV	pol	642-650	ALMPLYACI (SEQ. ID NO.: 131)	HLA-A*0201	129
HBV	env	378-387	LLPIFFCLWV (SEQ. ID NO.: 132)	HLA-A*0201	129
HBV	pol	538-546	YMDDVVLGA (SEQ. ID NO.: 133)	HLA-A*0201	129
HBV	env	250-258	LLLCLIFLL (SEQ. ID NO.: 134)	HLA-A*0201	130
HBV	env	260-269	LLDYQGMLPV (SEQ. ID NO.:135)	HLA-A*0201	130
HBV	env	370-379	SIVSPFIPLL (SEQ. ID NO.: 136)	HLA-A*0201	130
HBV	env	183-191	FLLTRILTI (SEQ. ID NO.: 137)	HLA-A*0201	130
HBV	cAg	88-96	YVNVNMGLK (SEQ. ID NO.: 138)	HLA-A* 1101	131
HBV	cAg	141-151	STLPETTVVRR (SEQ. ID NO.: 139)	HLA-A*3101	132
HBV	cAg	141-151	STLPETTVVRR (SEQ. ID NO.: 139)	HLA-A*6801	132
HBV	cAg	18-27	FLPSDFFPSV (SEQ, ID NO.: 126)	HLA-A*6801	127
HBV	sAg	28-39	IPQSLDSWWTSL (SEQ. ID NO.: 140)	H2-Ld	133
HBV	cAg	93-100	MGLKFRQL (SEQ. ID NO.: 141)	H2-Kb	134
HBV	preS	141-149	STBXQSGXQ (SEQ. ID NO.: 142)	HLA-A*0201	135
HCMV	gp B	618-628	FIAGNSAYEYV (SEQ. ID NO.: 143)	HLA-A*0201	124
HCMV	E1	978-989	SDEEFAIVAYTL (SEQ. ID NO.: 144)	HLA-B18	136
HCMV	pp65	397-411	DDVWTSGSDSDEEL V (SEQ. ID NO.: 145)	HLA-b35	137
HCMV	pp65	123-131	IPSINVHHY (SEQ. ID NO.: 146)	HLA-B*3501	136
HCMV	pp65	495-504	NLVPMVATVO (SEQ. ID NO.: 147)	HLA-A*0201	137
HCMV	pp65	415-429	RKTPRVTOGGAMA GA (SEQ. ID NO.: 148)	HLA-B7	137
HCV	MP	17-25	DLMGYIPLV (SEQ. ID NO.: 149)	HLA-A*0201	138
HCV	MP	63-72	LLALLSCLTV (SEQ. ID NO.: 15O)	HLA-A*0201	139

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HCV	MP	105-112	ILHTPGCV (SEQ. ID NO.: 151)	HLA-A*0201	139
HCV	env E	66-75	QLRRHIDLLV (SEQ. ID NO.: 152)	HLA-A*0201	139
HCV	env E	88-96	DLCGSVFLV (SEQ. ID NO.: 153)	HLA-A*0201	139
HCV	env E	172-180	SMVGNWAKV (SEQ. ID NO.: 154)	HLA-A*0201	139
HCV	NSI	308-316	HLIIQNIVDV (SEQ. ID NO.: 155)	HLA-A*0201	139
HCV	NSI	340-348	FLLLADARV (SEQ. ID NO.: 156)	HLA-A*0201	139
HCV	NS2	234-246	GLRDLAVAVEPVV (SEQ. ID NO.: 157)	HLA-A*0201	139
HCV	NSI	18-28	SLLAPGAKQNV (SEQ. ID NO.: 158)	HLA-A*0201	139
HCV	NSI	19-28	LLAPGAKQNV (SEQ. ID NO.: 159)	HLA-A*0201	139
HCV	NS4	192-201	LLFNILGGWV (SEQ. ID NO.: 160)	HLA-A*0201	129
HCV	NS3	579-587	YLVAYQATV (SEQ. ID NO.: 161)	HLA-A*0201	129
HCV	core protein	34-43	YLLPRRGPRL (SEQ. ID NO.: 162)	HLA-A*0201	129
HCV	MP	63-72	LLALLSCLTI (SEQ. ID NO.: 163)	HLA-A*0201	129
HCV	NS4	174-182	SLMAFTAAV (SEQ. ID NO.: 164)	HLA-A*0201	140
HCV	NS3	67-75	CINGVCWTV (SEQ. ID NO.: 165)	HLA-A*0201	140
HCV	NS3	163-171	LLCPAGHAV (SEQ. ID NO.: 166)	HLA-A*0201	141
HCV	NS5	239-247	ILDSFDPLV (SEQ, ID NO.: 167)	HLA-A*0201	141
HCV	NS4A	236-244	ILAGYGAGV (SEQ. ID NO.: 168)	HLA-A*0201	142
HCV	NS5	714-722	GLQDCTMLV (SEQ. ID NO.: 169)	HLA-A*0201	142
HCV	NS3	281-290	TGAPVTYSTY (SEQ. ID NO.: 170)	HLA-A*0201	143
HCV	NS4A	149-157	HMWNFISGI (SEQ. ID NO.: 171)	HLA-A*0201	144
HCV	NS5	575-583	RVCEKMALY (SEQ. ID NO.: 172)	HLA-A*0201-A3	145
HCV	NS1	238-246	TINYTIFK (SEQ. ID NO.: 173)	HLA-A*1101	145
HCV	NS2	109-116	YISWCLWW (SEQ. ID NO.: 174)	HLA-A23	145
HCV	core protein	40-48	GPRLGVRAT (SEQ. ID NO.: 175)	HLA-B7	145
HIV-1	gp120	380-388	SFNCGGEFF (SEQ. ID NO.: 176)	HLA-Cw*0401	185
HIV-1	RT	206-214	TEMEKEGKI	H2-Kk	186

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
			(SEQ. ID NO.: 177)		
HIV-1	p17	18-26	KIRLRPGGK	HLA-A*0301	187
			(SEQ. ID NO.: 178)		
HIV-1	P17	20-29	RLRPGGKKKY	HLA-A*0301	188
			(SEQ. ID NO.: 179)		
HIV- I	RT	325-333	AIFQSSMTK	HLA-A*0301	188
			(SEQ..ID NO.: 180)		
HIV-1	p17	84-92	TLYCVHQRI	HLA-A11	188
			(SEQ. ID NO.: 181)		
HIV-1	RT	508-517	IYQEPFKNLK	HLA-A11	188
			(SEQ. ID NO.: 182)		
HIV-1	p17	28-36	KYKLKHIVW	HLA-A24	188
			(SEQ. ID NO.: 183)		
HIV-1	gp120	53-62	LFCASDAKAY	HLA-A24	189
			(SEQ. ID NO.: 184)		
HIV-1	gagp24	145-155	QAISPRTLNAW	HLA-A25	188
			(SEQ. ID NO.: 185)		
HIV-1	gagp24	167-175	EVIPMFSAL	HLA-A26	188
			(SEQ. ID NO.: 186)		
HIV-1	RT	593-603	ETFYVDGAANR	HLA-A26	188
			(SEQ. ID NO.: 187)		
HIV-1	gp41	775-785	RLRDLLLIVTR	HLA-A31	190
			(SEQ. ID NO.: 188)		
HIV-1	RT	559-568	PIQKETWETW	HLA-A32	187
			(SEQ. ID NO.: 189)		
HIV-1	gpl20	419-427	RIKQIINMW	HLA-A32	187
			(SEQ. ID NO.: 190)		
HIV-1	RT	71-79	ITLWQRPLV	HLA-A*6802	188
			(SEQ. ID NO.: 191)		
HIV-1	RT	85-93	DTVLEEMNL	HLA-A*6802	188
			(SEQ. ID NO.: 192)		
HIV-1	RT	71-79	ITLWQRPLV	HLA-A*7401	188
			(SEQ. ID NO.: 193)		
HIV-1	gag p24	148-156	SPRTLNAWV	HLA-B7	188
			(SEQ. ID NO.: 194)		
HIV-1	gagp24	179-187	ATPQDLNTM	HLA-B7	188
			(SEQ. ID NO.: 195)		
HIV-1	gpl20	303-312	RPNNNTRKSI	HLA-B7	188
			(SEQ. ID NO.: 196)		
HIV-1	gp41	843-851	IPRRIRQGL	HLA-B7	188
			(SEQ. ID NO.: 197)		
HIV-1	p17	74-82	ELRSLYNTV	HLA-B8	188
			(SEQ. ID NO.: 198)		
HIV-1	nef	13-20	WPTVRERM	HLA-B8	188
			(SEQ. ID NO.: 199)		
HIV-1	nef	90-97	FLKEKGGL	HLA-B8	188
			(SEQ. ID NO.:200)		
HIV-1	gag p24	183-191	DLNTMLNTV	HLA-B14	191
			(SEQ. ID NO.:568)		
HIV-1	P17	18-27	KIRLRPGGKK	HLA-B27	188
			(SEQ. ID NO.:201)		
HIV-1	p17	19-27	IRLRPGGKK	HLA-B27	188
			(SEQ. ID NO.:202)		
HIV-1	gp41	791-799	GRRGWEALKY	HLA-B27	188

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HIV-1	nef	73-82	(SEQ. ID NO.:203) QVPLRPMTYK	HLA-B27	188
HIV-1	GP41	590-597	(SEQ. ID NO.:204) RYLKDQQL	HLA-B27	192
HIV-1	nef	105-114	(SEQ. ID NO.:205) RRQDILDLWI	HLA-B*2705	188
HIV-1	nef	134-141	(SEQ. ID NO.:206) RYPLTFGW	HLA-B*2705	188
HIV-1	p17	36-44	(SEQ. ID NO.:207) WASRELERF	HLA-B35	188
HIV-1	GAG P24	262-270	(SEQ. ID NO.:208) TVLDVGDAY	HLA-B35	188
HIV-1	gp120	42-52	(SEQ. ID NO.:209) VPVWKEATTTL	HLA-B35	188
HIV-1	P17	36-44	(SEQ. ID NO.:210) NSSKVSQNY	HLA-B35	193
HIV-1	gag p24	254-262	(SEQ. ID NO.:221) PPIPVGDIY	HLA-B35	193
HIV-1	RT	342-350	(SEQ. ID NO.:212) HPDIVIYQY	HLA-B35	193
HIV-1	gp41	611-619	(SEQ. ID NO.:213) TAVPWNASW	HLA-B35	194
HIV-1	gag	245-253	(SEQ. ID NO.:214) NPVPVGNIY	HLA-B35	193
HIV-1	nef	120-128	(SEQ. ID NO.:215) YFPDWQNYT	HLA-B37	188
HIV-1	gag p24	193-201	(SEQ. ID NO.:216) GHQAAMQML	HLA-B42	188
HIV-1	p17	20-29	(SEQ. ID NO.:217) RLRPGGKKKY	HLA-B42	188
HIV-1	RT	438-446	(SEQ. ID NO.:218) YPGIKVRQL	HLA-B42	188
HIV-1	RT	591-600	(SEQ. ID NO.:219) GAETFYVDGA	HLA-B45	188
HIV-1	gag p24	325-333	(SEQ. ID NO.:220) NANPDCKTI	HLA-B51	188
HIV-1	gag p24	275-282	(SEQ. ID NO.:221) RMYSPTSI	HLA-B52	188
HIV-1	gp120	42-51	(SEQ. ID NO.:222) VPVWKEATTT	HLA-B*5501	192
HIV-1	gag p24	147-155	(SEQ. ID NO.:223) ISPRTLNAW	HLA-B57	188
HIV-1	gag p24	240-249	(SEQ. ID NO.:224) TSTLQEQIGW	HLA-B57	188
HIV-1	gag p24	162-172	(SEQ. ID NO.:225) KAFSPEVIPMF	HLA-B57	188
HIV-1	gag p24	311-319	(SEQ. ID NO.:226) QASQEVKNW	HLA-B57	188
HIV-1	gag p24	311-319	(SEQ. ID NO.:227) QASQDVKNW	HLA-B57	188
HIV-1	nef	116-125	(SEQ. ID NO.:228) HTQGYFPDWQ (SEQ. ID NO.:229)	HLA-B57	188

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HIV-1	nef	120-128	YFPDWQNYT (SEQ. ID NO.:230)	HLA-B57	188
HIV-1	gag p24	240-249	TSTLQEQIGW (SEQ. ID NO.:231)	HLA-B58	188
HIV-1	p17	20-29	RLRPGGKKKY (SEQ. ID NO.:232)	HLA-B62	188
HIV-1	p24	268-277	LGLNKJVRMY (SEQ. ID NO.:233)	HLA-B62	188
HIV-1	RT	415-426	LVGKLNWASQIY (SEQ. ID NO.:234)	HLA-B62	188
HIV-1	RT	476-485	ILKEPVHGVY (SEQ. ID NO.:235)	HLA-B62	188
HIV-1	nef	117-127	TQGYFPDWQNY (SEQ. ID NO.:236)	HLA-B62	188
HIV-1	nef	84-91	AVDLSHFL (SEQ. ID NO.:237)	HLA-B62	188
HIV-1	gag p24	168-175	VIPMFSAL (SEQ. ID NO.:238)	HLA-Cw*0102	188
HIV-1	gp120	376-384	FNCGGEFFY (SEQ. ID NO.:239)	HLA-A29	196
HIV-1	gp120	375-383	SFNCGGEFF (SEQ. ID NO.:240)	HLA-B15	196
HIV-1	nef	136-145	PLTFGWCYKL (SEQ. ID NO.:241)	HLA-A*0201	197
HIV-1	nef	180-189	VLEWRFDSRL (SEQ. ID NO.:242)	HLA-A*0201	197
HIV-1	nef	68-77	FPVTPQVPLR (SEQ. ID NO.:243)	HLA-B7	197
HIV-1	nef	128-137	TPGPGVRYPL (SEQ. ID NO.:244)	HLA-B7	197
HIV-1	gag p24	308-316	QASQEVKNW (SEQ. ID NO.:245)	HLA-Cw*0401	521
HIV-1 IIIB	RT	273-282	VPLDEDFRKY (SEQ. ID NO.:246)	HLA-B35	181
HIV-1 IIIB	RT	25-33	NPDIVIYQY (SEQ. ID NO.:247)	HLA-B35	181
HIV-1 IIIB	gp41	557-565	RAIEAQAHL (SEQ. ID NO.:248)	HLA-B51	181
HIV-1 IIIB	RT	231-238	TAFTIPSI (SEQ. ID NO.:249)	HLA-B51	181
HIV- I IIIB	p24	215-223	VHPVHAGPIA (SEQ. ID NO.:250)	HLA-B*5501	181
HIV-1 IIIB	gp120	156-165	NCSFNISTSI (SEQ. ID NO.:251)	HLA-Cw8	181
HIV- I IIIB	gp120	241-249	CTNVSTVQC (SEQ. ID NO.:252)	HLA-Cw8	181
HIV-1 5F2	gp120	312-320	IGPGRAFHT (SEQ. ID NO.:253)	H2-Dd	198
HIV-1 5F2	pol	25-33	NPDIVIYQY (SEQ. ID NO.:254)	HLA-B*3501	199
HIV-15F2	pol	432-441	EPIVGAETFY (SEQ. ID NO.:255)	HLA-B*3501	199
HIV-1 5F2	pol	432-440	EPIVGAETF	HLA-B*3501	199

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
			(SEQ. ID NO.:256)		
HIV-1 5F2	pol	6-14	SPAIFQSSM	HLA-B*3501	199
			(SEQ. ID NO.:257)		
HIV-1 5F2	pol	59-68	VPLDKDFRKY	HLA-B*3501	199
			(SEQ. ID NO.:258)		
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B*3501	199
			(SEQ. ID NO.:259)		
HIV-1 5F2	nef	69-79	RPQVPLRPMTY	HLA-B*3501	199
			(SEQ. ID NO.:260)		
HIV-1 5F2	nef	66-74	FPVRPQVPL	HLA-B*3501	199
			(SEQ. ID NO.:261)		
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B*3501	199
			(SEQ. ID NO.:262)		
HIV-1 5F2	env	7-15	RPIVSTQLL	HLA-B*3501	199
			(SEQ. ID NO.:263)		
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B51	199
			(SEQ. ID NO.:264)		
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B51	199
			(SEQ. ID NO.:265)		
HIV-1 5F2	gagp24	199-207	AMQMLKETI	H2-Kd	198
			(SEQ. ID NO.:266)		
HIV-2	gagp24	182-190	TPYDrNQML	HLA-B*5301	200
			(SEQ. ID NO.:267)		
HIV-2	gag	260-269	RRWIQLGLQKV	HLA-B*2703	188
			(SEQ. ID NO.:268)		
HIV-1 5F2	gp41	593-607	GIWGCSGKLICTTA V	HLA-B17	201
			(SEQ. ID NO.:269)		
HIV-1 5F2	gp41	753-767	ALIWEDLRSLCLFSY	HLA-B22	201
			(SEQ. ID NO.:270)		
HPV 6b	E7	21-30	GLHCYEQLV	HLA-A*0201	202
			(SEQ. ID NO.:271)		
HPV 6b	E7	47-55	PLKQHFQIV	HLA-A*0201	202
			(SEQ. ID NO.:272)		
HPV11	E7	4-12	RLVTLKDIV	HLA-A*0201	202
			(SEQ. ID NO.:273)		
HPV16	E7	86-94	TLGIVCPIC	HLA-A*0201	129
			(SEQ. ID NO.:274)		
HPV16	E7	85-93	GTLGIVCPI	HLA-A*0201	129
			(SEQ. ID NO.:275)		
HPV16	E7	12-20	MLDLQPETT	HLA-A*0201	129
			(SEQ. ID NO.:276)		
HPV16	E7	11-20	YMLDLQPETT	HLA-A*0201	203
			(SEQ. ID NO.:277)		
HPVl6	E6	15-22	RPRKLPQL	HLA-B7	204
			(SEQ. ID NO.:278)		
HPV16	E6	49-57	RAHYNIVTF	HW-Db	205
			(SEQ. ID NO.:279)		
HSV	gp B	498-505	SSIEFARL	H2-Kb	206
			(SEQ. ID NO.:280)		
HSV-1	gp C	480-488	GIGIGVLAA	HLA-A*0201	104
			(SEQ. ID NO.:281)		
HSV-1	ICP27	448-456	DYATLGVGV	H2-Kd	207
			(SEQ. ID NO.:282)		

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
HSV-1	ICP27	322-332	LYRTFAGNPRA (SEQ. ID NO.:283)	H2-Kd	207
HSV-1	UL39	822-829	QTFDFGRL (SEQ. ID NO.:284)	H2-Kb	208
HSV-2	gpC	446-454	GAGIGVAVL (SEQ. ID NO.:285)	HLA-A*0201	104
HLTV-1	TAX	11-19	LLFGYPVYV (SEQ. ID NO.:286)	HLA-A*0201	209
Influenza	MP	58-66	GILGFVFTL (SEQ. ID NO.:287)	HLA-A*0201	68,169,209,210, 211
Influenza	MP	59-68	ILGFVFTLTV (SEQ. ID NO.:288)	HLA-A*0201	168,212,213
Influenza	NP	265-273	ILRGSVAHK (SEQ, ID NO.:289)	HLA-A3	214
Influenza	NP	91-99	KTGGPIYKR (SEQ. ID NO.:290)	HLA-A*6801	215,216
Influenza	NP	380-388	ELRSRYWAI (SEQ. ID NO.:291)	HLA-B8	217
Influenza	NP	381-388	LRSRYWAI (SEQ. ID NO.:292)	HLA-B*2702	218
Influenza	NP	339-347	EDLRVLSFI (SEQ. ID NO.:293)	HLA-B*3701	219
Influenza	NSI	158-166	GEISPLPSL (SEQ. ID NO.:294)	HLA-B44	220
Influenza	NP	338-346	FEDLRVLSF (SEQ. ID NO.:295)	HLA-B44	220
Influenza	NSI	158-166	GEISPLPSL (SEQ. ID NO.:294)	HLA-B*4402	220
Influenza	NP	338-346	FEDLRVLSF (SEQ. ID NO.:295)	HLA-B*4402	220
Influenza	PBI	591-599	VSDGGPKLY (SEQ. ID NO.:296)	HLA-Al	214,29
Influenza A	NP	44-52	CTELKLSDY (SEQ. ID NO.:297)	HLA-Al	29
Influenza	NSI	122-130	AIMDKNIIL (SEQ. ID NO.:298)	HLA-A*0201	221
Influenza A	NSI	123-132	IMDKNIILKA (SEQ. ID NO.:299)	HLA-A*0201	221
Influenza A	NP	383-391	SRYWAIRTR (SEQ. ID NO.:300)	HLA-B*2705	160,184
Influenza A	NP	147-155	TYQRTRALV (SEQ. ID NO.:301)	H2-Kd	222,223
Influenza A	HA	210-219	TYVSVSTSTL (SEQ. ID NO.302)	H2-Kd	224,225
Influenza A	HA	518-526	IYSTVASSL (SEQ. ID NO.303)	H2-Kd	224
Influenza A	HA	259-266	FEANGNLI (SEQ. ID NO.:304)	H2-Kk	226,227,228
Influenza A	HA	10-18	IEGGWTGMl (SEQ. ID NO.:305)	H2-Kk	226,227,228
Influenza A	NP	50-57	SDYEGRLI (SEQ. ID NO..306)	H2-Kk	229,230
Influenza a	NSI	152-160	EEGAIVGEI	H2-Kk	231

Table I: Viral epitopes on MHC class [redacted] molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
Influenza A34	NP	336-374	(SEQ. ID NO.:307) ASNENMETM	H2Db	168,222,219
Influenza A68	NP	366-374	(SEQ. ID NO.:308) ASNENMDAM	H2Db	232
Influenza B	NP	85-94	(SEQ. ID NO.:309) KLGEFYNQMM	HLA-A*0201	233
Influenza B	NP	85-94	(SEQ. ID NO.:310) KAGEFYNQMM	HLA-A*0201	234
Influenza JAP	HA	204-212	(SEQ. ID NO.:311) LYQNVGTYV	H2Kd	235
Influenza JAP	HA	210-219	(SEQ. ID NO.312) TYVSVGTSTL	H2-Kd	225
Influenza JAP	HA	523-531	(SEQ. ID NO.:313) VYQILATYA	H2-Kd	235
Influenza JAP	HA	529-537	(SEQ. ID NO.314) IYATVAGSL	H2-Kd	235
Influenza JAP	HA	210-219	(SEQ. ID NO.315) TYVSVGTSTI(L>I)	H2-Kd	236
Influenza JAP	HA	255-262	(SEQ. ID NO.:316) FESTGNLI	H2-Kk	237
JHMV	cAg	318-326	(SEQ. ID NO.:317) APTAGAFFF	H2-Ld	238
LCMV	NP	118-126	(SEQ. ID NO.:318) RPQASGVYM	H2-Ld	239-240
LCMV	NP	396-404	(SEQ. ID NO.319) FQPQNGQFI	H2-Db	241
LCMV	GP	276-286	(SEQ. ID NO.320) SGVENPGGYCL	H2-Db	242
LCMV	GP	33-42	(SEQ. ID NO.:321) KAVYNFATCG	H2-Db	243,244
MCMV	pp89	168-176	(SEQ. ID NO.:322) YPHFMPTNL	H2-Ld	245
MHV	spike protein	510-518	(SEQ. ID NO.323) CLSWNGPHL	H2-Db	248
MMTV	env gp 36	474-482	(SEQ. ID NO.324) SFAVATTAL	H2-Kd	246
MMTV	gag p27	425-433	(SEQ. ID NO.:325) SYETFISRL	H2-Kd	246
MMTV	env gp73	544-551	(SEQ. ID NO.:326) ANYDFICV	H2-Kb	247
MuLV	env p15E	574-581	(SEQ. ID NO.:327) KSPWFTTL	H2-Kb	249,250
MuLV	env gp70	189-196	(SEQ. ID NO.:328) SSWDFITV	H2-Kb	251,Sijts et al. Submitted
MuLV	gag 75K	75-83	(SEQ. ID NO.:329) CCLCLTVFL	H2-Db	252
MuLV	env gp70	423-431	(SEQ. ID NO.:330) SPSYVYHQF	H2Ld	253
MV	F protein	437-447	(SEQ. ID NO.:331) SRRYPDAVYLH	HLA-B*2705	254
Mv	F protein	438-446	(SEQ. ID NO.:332) RRYPDAVYL	HLA-B*2705	255

Table I: Viral epitopes on MHC class I molecules

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule	Ref.
Mv	NP	281-289	(SEQ. ID NO.333) YPALGLHEF	H2-Ld	256
Mv	HA	343-351	(SEQ. ID NO.:334) DPVIDRLYL	H2-Ld	257
MV	HA	544-552	(SEQ. ID NO.335) SPGRSFSYF	H2-Ld	257
Poliovirus	VP1	111-118	(SEQ. ID NO.:336) TYKDTVQL	H2-kd	258
Poliovirus	VP1	208-217	(SEQ. ID NO.:337) FYDGFSKVPL	H2-Kd	258
Pseudorabies virus gp	G111	455-463	(SEQ. ID NO.:338) IAGIGILAI	HLA-A*0201	104
Rabiesvirus	NS	197-205	(SEQ. ID NO.:339) VEAEIAHQI	H2-Kk	227-227
Rotavirus	VP7	33-40	(SEQ. ID NO.:340) llYRFLLl	H2-Kb	259
Rotavirus	VP6	376-384	(SEQ. ID NO.:341) VGPVFPPGM	H2-Kb	260
Rotavirus	VP3	585-593	(SEQ. ID NO.:342) YSGYIFRDL	H2-Kb	260
RSV	M2	82-90	(SEQ. ID NO.:343) SYIGSINNI	H2-Kd	261
SIV	gagp11C	179-190	(SEQ. ID NO.:344) EGCTPYDTNQML	Mamu-A*01	266
SV	NP	324-332	(SEQ. ID NO.:345) FAPGNYPAL	H2-Db	262
SV	NP	324-332	(SEQ. ID NO.:346) FAPCTNYPAL	H2-Kb	263,264,265
SV40	T	404-411	(SEQ. ID NO.:346) VVYDFLKC	H2-Kb	267
SV40	T	206-215	(SEQ. ID NO.:347) SAINNYAQKL	H2-Db	268,269
SV40	T	223-231	(SEQ. ID NO.:348) CKGVNKEYL	H2-Db	268,269
SV40	T	489-497	(SEQ. ID NO.349) QGINNLDNL	H2-Db	268,269
SV40	T	492-500 (501)	(SEQ. ID NO.:350) NNLDNLRDY(L)	H2-Db	270
SV40	T	560-568	(SEQ. ID NO.:351) SEFLLEKRI	H2-Kk	271
VSV	NP	52-59	(SEQ. ID NO.352) RGYVYQGL (SEQ. ID NO.:353)	H2-Kb	272

Table II sets forth antigens identified from various protein sources. The Table is extracted from Table 4.2 in the Rammensee book with the references in Table H being the same as the references in the Rammensee Table 4.2.

TABLE II

HLA Class I Motifs

HLA-A1	Position (Antigen)	Source	Ref.
T cell epitopes	EADPTGHSY	MAGE-1 161-169	27,28
	(SEQ. ID NO.:354)		
	VSDGGPNLY	Influenza A PB 1591-599	21,23
	(SEQ. ID NO.:355)		
	CTELKLSDY	Influenza A NP 44-52	23
	(SEQ. ID NO.:356)		
	EVDPIGHLY	MAGE-3 168-176	29,30
	(SEQ. ID NO.:357)		
HLA-A201	MLLSVPLLLG	Calreticulin signal sequence I-10	34,35,36,37
	(SEQ, ID NO.:358)		
	STBXQSGXQ	HBV PRE-S PROTEIN 141-149	43
	(SEQ. ID NO.:359)		
	YMDGTMSQV	Tyrosinase 369-377	45
	(SEQ, ID NO.:360)		
	ILKEPVHGV	HIV- I RT 476-484	4,31,47
	(SEQ. ID NO.:361)		
	LLGFVFTLTV	Influenza MP 59-68	4,39
	(SEQ. ID NO,:362)		
	LLFGYPVYVV	HTLV-1 tax 11-19	40
	(SEQ. ID NO.:363)		
	GLSPTVWLSV	HBV sAg 348-357	48
	(SEQ. ID NO.:364)		
	WLSLLVPFV	HBV sAg 335-343	49,50.51
	(SEQ. ID NO.:365)		

TABLE II
HLA Class I Motifs

	FLPSDFFPSV	HBV cAg 18-27	52
	(SEQ. ID NO.:366)		
	C L G 0 L L T M V	EBV LMP-2 426-434	48
	(SEQ. ID NO.:367)		
	FLAGNSAYEYV	HCMV gp 618-628B	53
	(SEQ. ID NO.:368)		
	KLGEFYNQMM	Influenza BNP 85-94	54
	(SEQ. ID NO.:369)		
	KLVALGINAV	HCV-1 NS3 400-409	55
	(SEQ. ID NO,:370)		
	DLMGYIPLV	HCV MP 17-25	56
	(SEQ. ID NO.:37 1)		
	RLVTLKDIV	HPV 11 EZ 4-12	34,35
	(SEQ. ID NO.:372)		
	MLLAVLYCL	Tyrosinase 1-9	57,58,59,68
	(SEQ. ID NO-373)		
	AAGIGILTV	Melan A\Mart-127-35	60
	(SEQ. ID NO.:374)		
	YLEPGPVTA	Pmel 17/gp 100 480-488	61
	(SEQ. ID NO.:375)		
	ILDGTATLRL	Pmel 17/ gp 100 457-466	62
	(SEQ. ID NO.:376)		
	LLDGTATLRL	Pmel gplOO 457-466	62
	(SEQ. ID NO.:377)		
	ITDQVPFSV	Pmel gp 100 209-217	62
	(SEQ. ID NO.:378)		
	KTWGQYWQV	Pmel gp 100 154-162	62
	(SEQ. ID NO.:379)		
	TITDQVPFSV	Pmel gp 100 208-217	62
	(SEQ. ID NO.:380)		
	AFHIIVAREL	HIV- I nef 190-198	63
	(SEQ. ID NO.:381)		

TABLE II
HLA Class I Motifs

	YLNKIQNSL	P. falciparum CSP 334-342	64
	(SEQ. ID NO.:382)		
	MMRKLAILSV	P. falciparum CSP 1 -10	64
	(SEQ. ID NO.:383)		
	KAGEFYNQMM	Influenza BNP 85-94	65
	(SEQ. ID NO.:384)		
	NIAEGLRAL	EBNA-1 480-488	66
	(SEQ. ID NO.:385)		
	NLRRGTALA	EBNA-1 519-527	66
	(SEQ. ID NO.:386)		
	ALAIPQCRL	EBNA-1 525-533	66
	(SEQ. ID NO.:387)		
	VLKDAIKDL	EBNA-1 575-582	66
	(SEQ. ID NO.:388)		
	FMVFLQTHI	EBNA-1 562-570	66
	(SEQ. ID NO.:389)		
	HLIVDTDSL	EBNA-2 15-23	66
	(SEQ. ID NO.:390)		
	SLGNPSLSV	EBNA-2 22-30	66
	(SEQ. ID NO.391)		
	PLASAMRML	EBNA-2 126-134	66
	(SEQ. ID NO.392)		
	RMLWMANYI	EBNA-2 132-140	66
	(SEQ. ID NO.:393)		
	MLWMANYIV	EBNA-2 133-141	66
	(SEQ. ID NO.:394)		
	ILPQGPQTA	EBNA-2 151-159	66
	(SEQ. ID NO.:395)		
	PLRPTAPTTI	EBNA-2 171-179	66
	(SEQ, ID NO.:396)		
	PLPPATLTV	EBNA-2 205-213	66
	(SEQ. ID NO.:397)		

TABLE II
HLA Class I Motifs

	R M H L P V L H V	EBNA-2 246-254	66
	(SEQ. ID NO.397)		
	PMPLPPSQL	EBNA-2 287-295	66
	(SEQ. ID NO.:399)		
	QLPPPAAPA	EBNA-2 294-302	66
	(SEQ. ID NO.:400)		
	SMPELSPVL	EBNA-2 381-389	66
	(SEQ. ID NO.:401)		
	DLDESWDYI	EBNA-2 453-461	66
	(SEQ. ID NO.:402)		
	P L P C V L W P VV	BZLFl 43-51	66
	(SEQ. ID NO.:403)		
	SLEECDSEL	BZLFl 167-175	66
	(SEQ, ID NO.:404)		
	EIKRYKNRV	BZLFI 176-184	66
	(SEQ. ID NO.:405)		
	QLLQFIYREV	BZLF1 195-203	66
	(SEQ. ID NO.:406)		
	LLQHYREVA	BZLFI 196-204	66
	(SEQ. ID NO.:407)		
	LLKQMCPSL	BZLFI 217-225	66
	(SEQ. ID NO.:408)		
	SIIPRTPDV	BZLFI 229-237	66
	(SEQ. ID NO.:409)		
	AIMDKNIIL	Influenza A NS1 122-130	67
	(SEQ. ID NO.:410)		
	IMDKNIILKA	Influenza A NS1 123-132	67
	(SEQ. ID NO.411)		
	LLALLSCLTV	HCV MP 63-72	69
	(SEQ. ID NO.:412)		
	ILHTPGCV	HCV MP 105-112	69
	(SEQ. ID NO.:413)		

TABLE II
HLA Class I Motifs

	QLRRHIDLLV	HCV env E 66-75	69
	(SEQ, ID NO.:414)		
	DLCGSVFLV	HCV env E 88-96	69
	(SEQ. ID NO.:415)		
	SMVGNWAKV	HCV env E 172-180	69
	(SEQ. ID NO.:416)		
	HLHQNIVDV	HCV NSI 308-316	69
	(SEQ. ID NO.:417)		
	FLLLADARV	HCV NSI 340-348	69
	(SEQ. ID NO.:418)		
	GLRDLAVAVEPVV	HCV NS2 234-246	69
	(SEQ. ID NO.:419)		
	SLLAPGAKQNV	HCV NS1 18-28	69
	(SEQ. ID NO.:420)		
	LLAPGAKQNV	HCV NS1 19-28	69
	(SEQ. ID NO.:421)		
	FLLSLGIHL	HBV pol 575-583	70
	(SEQ. ID NO.:422)		
	SLYADSPSV	HBV pol 816-824	70
	(SEQ. ID NO.:423)		
	GLSRYVARL	HBV POL 455-463	70
	(SEQ. ID NO.:424)		
	KIFGSLAFL	HER-2 369-377	71
	(SEQ. ID NO.:425)		
	ELVSEFSRM	HER-2 971-979	71
	(SEQ. ID NO.:426)		
	KLTPLCVTL	HIV- I gp 160 120-128	72
	(SEQ. ID NO.:427)		
	SLLNATDIAV	HIV- I GP 160 814-823	72
	(SEQ. ID NO.:428)		
	VLYRYGSFSV	Pmel gpl00 476-485	62
	(SEQ. ID NO.:429)		

TABLE II
HLA Class I Motifs

	YIGEVLVSV	Non-filament forming class I myosin family (HA-2)**	73
	(SEQ. ID NO.:430)		
	LLFNILGGWV	HCV NS4 192-201	74
	(SEQ. ID NO.:431)		
	LLVPFVQWFW	HBV env 338-347	74
	(SEQ. ID NO.:432)		
	ALMPLYACI	HBV pol 642-650	74
	(SEQ. ID NO.:433)		
	YLVAYQATV	HCV NS3 579-587	74
	(SEQ. ID NO.:434)		
	TLGIVCPIC	HIPV 16 E7 86-94	74
	(SEQ. ID NO.:435)		
	YLLPRRGPRL	HCV core protein 34-43	74
	(SEQ. ID NO.:436)		
	LLPIFFCLWV	HBV env 378-387	74
	(SEQ. ID NO.:437)		
	YMDDVVLGA	HBV Pol 538-546	74
	(SEQ. ID NO.:438)		
	GTLGIVCPI	HPV16 E7 85-93	74
	(SEQ. ID NO.:439)		
	LLALLSCLTI	HCV MP 63-72	74
	(SEQ. ID NO.:440)		
	MLDLQPETT	HPV 16 E7 12-20	74
	(SEQ. ID NO.:441)		
	SLMAFTAAV	HCV NS4 174-182	75
	(SEQ. ID NO.:442)		
	CINGVCWTV	HCV NS3 67-75	75
	(SEQ. ID NO.:443)		
	VMNILLQYVV	Glutarnic acid decarboxylase 114-123	76
	(SEQ. ID NO.:444)		

TABLE II
HLA Class I Motifs

	ILTVILGVL	Melan A/Mart- 32-40	77
	(SEQ. ID NO.:445)		
	FLWGPRALV	MAGE-3 271-279	78
	(SEQ. ID NO.:446)		
	L L C P A G H A V	HCV NS3 163-171	54
	(SEQ. ID NO.:447)		
	ILDSFDPLV	HCV NSS 239-247	54
	(SEQ. ID NO.:448)		
	LLLCLIFLL	HBV env 250-258	79
	(SEQ. ID NO.:449)		
	LIDYQGMLPV	HBV env 260-269	79
	(SEQ. ID NO.:450)		
	SIVSPFIPLL	HBV env 370-379	79
	(SEQ.ID NO.:451)		
	FLLTRILTI	HBV env 183-191	80
	(SEQ, ID NO.:452)		
	HLGNVKYLV	P. faciparum TRAP 3-11	81
	(SEQ, ID NO.:453)		
	GIAGGLALL	P. faciparum TRAP 500-508	81
	(SEQ. ID NO.:454)		
	ILAGYGAGV	HCV NS S4A 236-244	82
	(SEQ. ID NO.:455)		
	GLQDCTMLV	HCV NS5 714-722	82
	(SEQ. ID NO.:456)		
	TGAPVTYSTY	HCV NS3 281-290	83
	(SEQ. ID NO.:457)		
	VIYQYMDDLV	HIV-1RT 179-187	84
	(SEQ, ID NO.:458)		
	VLPDVFIRCV	N-acetylglucosaminyltransferase V Gnt-V intron	85
	(SEQ, ID NO.:459)		

TABLE II
HLA Class I Motifs

	VLPDVFIRC	N-acetylglucosaminyltransferase V Gnt-V intron	85
	(SEQ. ID NO.:460)		
	AVGIGIAVV	Human CD9	86
	(SEQ. ID NO.:461)		
	LVVLGLLAV	Human glutamyltransferase	86
	(SEQ, ID NO.:462)		
	ALGLGLLPV	Human G protein coupled receptor	86
	(SEQ, ID NO.:463) 164-172		
	GIGIGVLAA	HSV- I gp C 480-488	86
	(SEQ. ID NO.:281)		
	GAGIGVAVL	HSV-2 gp C 446-454	86
	(SEQ. ID NO.:464)		
	IAGIGILAI	Pseudorabies gpGIN 455-463	86
	(SEQ. ID NO.:465)		
	LIVIGILIL	Adenovirus 3 E3 9kD 30-38	86
	(SEQ. ID NO.:466)		
	LAGIGLIAA	S. Lincolnensis ImrA	86
	(SEQ. ID NO.:467)		
	VDGIGILTI	Yeast ysa-1 77-85	86
	(SEQ. ID NO.:468)		
	GAGIGVLTA	B. polymyxa, βcndoxylanase 149-	86
	(SEQ. ID NO.:469) 157		
	AAGIGIIQI	E. coli methionine synthase 590-598	86
	(SEQ. ID NO.:470)		
	QAGIGILLA	E. coli hypothetical protein 4-12	86
	(SEQ. ID NO.:47 1)		
	KARDPHSGHFV	CDK4wl 22.32	87

TABLE II
HLA Class I Motifs

	(SEQ. ID NO.:472)		
	KACDPI-ISGIIFV	CDK4-R24C 22-32	87
	(SEQ. ID NO.:473)		
	ACDPFISGHFV	CDK4-R24C 23-32	87
	(SEQ. ID NO.:474)		
	SLYNTVATL	HIV- I gag p 17 77-85	99
	(SEQ. ID NO.:475)		
	ELVSEFSRV	HER-2, m>V substituted 971-979	89
	(SEQ. ID NO.:476)		
	RGPGRAFVTI	HIV- I gp 160 315-329	90
	(SEQ. ID NO.:477)		
	HMWNFISGI	HCV NS4A 149-157	91
	(SEQ. ID NO.:478)		
	NLVPMVATVQ	HCMV pp65 495-504	92
	(SEQ. ID NO.:479)		
	GLHCYEQLV	HPV 6b E7 21-30	93
	(SEQ. ID NO,:480)		
	PLKQHFQIV	HPV 6b E7 47-55	93
	(SEQ. ID NO.:481)		
	LLDFVRFMGV	EBNA-6 284-293	95
	(SEQ. ID NO.:482)		
	AIMEKNIML	Influenza Alaska NS 1 122-130	67
	(SEQ. ID NO.:483)		
	YLKTIQNSL	P. falciparum cp36 CSP	96
	(SEQ. ID NO.:484)		
	YLNKIQNSL	P. falciparum cp39 CSP	96
	(SEQ. ID NO.:485)		
	YMLDLQPETT	HPV 16 E7 11-20*	97
	(SEQ, ID NO.:486)		
	LLMGTLGIV	HPV16 E7 82-90**	97
	(SEQ. ID NO.:487)		

TABLE II
HLA Class I Motifs

	TLGIVCPI	HPV 16 E7 86-93	97
	(SEQ. ID NO.:488)		
	TLTSCNTSV	HIV-1 gp120 197-205	98
	(SEQ. ID NO.:489)		
	KLPQLCTEL	HPV 16 E6 18-26	97
	(SEQ. ID NO.:490)		
	TIHDIILEC	HPV16 E6 29-37	97
	(SEQ. ID NO.:491)		
	LGIVCPICS	HPV16 E7 87-95	97
	(SEQ. ID NO.:492)		
	VILGVLLLI	Melan A/Mart-1 35-43	68
	(SEQ. ID NO.:493)		
	ALMDKSLHV	Melan A/Mart- 1 56-64	68
	(SEQ. ID NO.:494)		
	GILTVILGV	Melan A/Mart- 1 31-39	68
	(SEQ. ID NO.:495)		
T cell epitopes	MINAYLDKL	P. Falciparum STARP 523-531	81
	(SEQ. ID NO.:496)		
	AAGIGILTV	Melan A/Mart- 127-35	100
	(SEQ. ID NO.:497)		
	FLPSDFFPSV	HBV cAg 18-27	51
	(SEQ. ID NO,:498)		
Motif unknown	SVRDRLARL	EBNA-3 464-472	101
T cell epitopes	(SEQ. ID NO.:499)		
T cell epitopes	AAGIGILTV	Melan A/Mart-1 27-35	100
	(SEQ. ID NO.:497)		
	FAYDGKDYI	Human MHC I-ot 140-148	99
	(SEQ. ID NO.:500)		
T cell epitopes	AAGIGILTV	Melan A/Mart-1 27-35	100
	(SEQ, ID NO.:497)		
	FLPSDFFPSV	HBV cAg 18-27	51

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TABLE II
HLA Class I Motifs

	(SEQ. ID NO.:498)		
Motif unknown	AAGIGILTV	Meland A/Mart-1 27-35	100
T cell epitopes	(SEQ. ID NO.:497)		
	FLPSDFFPSV	HBV cAg 18-27	51
	(SEQ. ID NO.:498)		
	AAGIGILTV	Melan A/Mart-1 27-35	100
	(SEQ. ID NO.:497)		
	ALLAVGATK	Pmel17 gp 100 17-25	107
	(SEQ. ID NO.:501)		
T cell epitopes	R L R D L L L I V T R	HIV-1 gp41 768-778	108
	(SEQ. ID NO.:502)		
	QVPLRPMTYK	HIV-1 nef 73-82	109
	(SEQ. ID NO.:503)		
	TVYYGVPVWK	HIV-1 gp120-36-45	110
	(SEQ. ID NO.:504)		
	RLRPGGKKK	HIV- 1 gag p 17 20-29	110
	(SEQ. ID NO.:505)		
	ILRGSVAHK	Influenza NP 265-273	21
	(SEQ. ID NO,:506)		
	RLRAEAGVK	EBNA-3 603-611	111
	(SEQ. ID NO.:507)		
	RLRDLLLIVTR	HIV-1 gp4l 770-780	112
	(SEQ. ID NO.:502)		
	VYYGVPVWK	HIV- I GP 120 38-46	113
	(SEQ. ID NO.:508)		
	RVCEKMALY	HCV NS5 575-583	114
	(SEQ. ID NO.:509)		
Motif unknown	KIFSEVTLK	Unknown; muta melanoma peptide ted (p I 83L) 175-183	Wolfel et al., pers. Comm.
T cell epitope	(SEQ, ID NO.:510)		
	YVNVNMGLK*	HBV cAg 88-96	116
	(SEQ. ID NO.:511)		

TABLE II
HLA Class I Motifs

T cell epitopes	IVTDFSVIK	EBNA-4 416-424	115,117
	(SEQ. ID NO.:512)		
	ELNEALELK	P53 343-351	115
	(SEQ, ID NO.:513)		
	VPLRPMTYK	HIV- 1 NEF 74-82	115
	(SEQ. ID NO.:514)		
	AIFQSSMTK	HIV- I gag p24 325-333	115
	(SEQ. ID NO.:515)		
	QVPLRPMTYK	HIV-1 nef 73-82	118
	(SEQ. ID NO.:516)		
	TINYTIFK HCV	NSI 238-246	114
	(SEQ. ID NO.:517)		
	AAVDLSHFLKEK	HIV-1 nef 83-94	120
	(SEQ. ID NO.:518)		
	ACQ G V G G P G G H K	HIV-1 I I 1B p24 349-359	122
	(SEQ, ID NO,:519)		
HLA-A24	S Y L D S G I H F*	β-catenin, mutated (proto-onocogen) 29-37	123
	(SEQ, ID NO.:520)		
T cell epitopes	RYLKDQQLL	HIV GP 41 583-591	124
	(SEQ. ID NO.:521)		
	AYGLDFYIL	P15 melanoma Ag 10- 18	125
	(SEQ. ID NO.:522)		
	AFLPWHRLFL	Tyrosinase 206-215	126
	(SEQ. ID NO.:523)		
	AFLPWHRLF	Tyrosinase 206-214	126
	(SEQ. ID NO.:524)		
	RYSIFFDY	Ebna-3 246-253	101
	(SEQ. ID NO.:525)		
T cell epitope	ETINEEAAEW	HIV- 1 gag p24 203-212	127

TABLE II
HLA Class I Motifs

	(SEQ. ID NO.:526)		
T cell epitopes	STLPETTVVRR	HBV cAg 141 -151	129
	(SEQ. ID NO,:527)		
	MSLQRQFLR	ORF 3P-gp75 294-321 (bp)	130
	(SEQ, ID NO.:528)		
	LLPGGRPYR	TRP (tyrosinase rel.) 197-205	131
	(SEQ. ID NO.:528)		
T cell epitope	IVGLNKIVR	HIV gag p24 267-267-275	132, 133
	(SEQ. ID NO.:530)		
	AAGIGILTV	Melan A/Mart- 127 35	100
	(SEQ. ID NO.:53 1)		

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Table III sets forth additional antigens useful in the invention that are available from the Ludwig Cancer Institute. The Table refers to patents in which the identified antigens can be found and as such are incorporated herein by reference. TRA refers to the tumor-related antigen and the LUD No. refers to the Ludwig Institute number.

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Table III

TRA	LUD No.	Patent No.	Date Patent Issued	Peptide (Antigen)	HLA
MAGE-4	5293	5,405,940	11 April 1995	EVDPASNTY (SEQ. ID NO.:532)	HLA-Al
MAGE-41	5293	5,405,940	11 April 1995	EVDPTSNTY (SEQ ID NO:533)	HLA-A I
MAGE-5	5293	5,405,940	11 April 1995	EADPTSNTY (SEQ ID NO:534)	HLA-A I
MAGE-51	5293	5,405,940	11 April 1995	EADPTSNTY (SEQ ID NO:534)	HLA-A I
MAGE-6	5294	5,405,940	11 April 1995	EVDPIGHVY (SEQ ID NO:535)	HLA-Al
	5299.2	5,487,974	30 January 1996	MLLAVLYCLL (SEQ ID NO:536)	HLA-A2
	5360	5,530,096	25 June 1996	MLLAVLYCL (SEQ ID NO:537)	HLA-B44
Tyrosinase	5360.1	5,519,117	21 May 1996	SEIWRDIDFA (SEQ ID NO:538) SEIWRDIDF (SEQ ID NO:539)	HLA-B44
Tyrosinase	5431	5,774,316	28 April 1998	XEIWRDIDF (SEQ ID NO:540)	HLA-B44
MAGE-2	5340	5,554,724	10 September 1996	STLVEVTLGEV (SEQ ID NO:541) LVEVTLGEV (SEQ ID NO:542) VIFSKASEYL (SEQ ID NO:543) IIVLAIIAI (SEQ ID NO:544) KIWEELSMLEV (SEQ ID NO:545)	HLA-A2

(Continued)

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Table III

TRA	LUD No.	Patent No.	Date Patent Issued	Peptide (Antigen)	HLA
				LIETSYVKV	
				(SEQ ID NO:546)	
	5327	5,585,461	17 December 1996	FLWGPRALV	HLA-A2
				(SEQ ID NO: 547)	
				TLVEVTLGEV	
				(SEQ ID NO:548)	
				ALVETSYVKV	
				(SEQ ID NO:549)	
MAGE-3	5344	5,554,506	10 September 1996	KIWEELSVL	HLA-A2
				(SEQ ID NO:550)	
MAGE-3	5393	5,405,940	11 April 1995	EVDPIGHLY	HLA-Al
				(SEQ ID NO:551)	
MAGE	5293	5,405,940	11 April 1995	EXDX5Y	HLA-Al
				(SEQ. ID NO.:552)	
				(but not EADPTGHSY)	
				(SEQ. ID NO.:553)	
				E (A/V) D X5 Y	
				(SEQ. ID NO.:554)	
				E (A/V) D P X4 Y	
				(SEQ. ID NO.:555)	
				E (A/V) D P (I/A/T) X3 Y	
				(SEQ. ID NO.:556)	
				E (A/V) D P (I/A/T) (G/S) X2 Y	
				(SEQ. ID NO.:557)	
				E (A/V) D P (I/A/T) (G/S) (H/N) X Y	
				E (A/V) DP (I/A/T) (G/S) (H/N) (L/T/V) Y	
				(SEQ. 11) NO.:559)	
MAGE-1	5361	5,558.995	24 September 1996	ELHSAYGEPRKLLTQD	HLA-C Clone 10
				(SEQ ID NO:560)	
				EHSAYGEPRKLL	
				(SEQ ID NO:561)	
				SAYGEPRKL	
				(SEQ ID NO:562)	
MAGE-1	5253.4	TBA	TBA	EADPTGHSY	HLA-A I
				(SEQ ID NO:563)	

Table III

TRA	LUD No.	Patent No.	Date Patent Issued	Peptide (Antigen)	HLA
				(SEQ ID NO:564)	Clone 10
				MAARAVFLALSAQLLQ	HLA-C
				(SEQ ID NO:565)	Clone 10
				AARAVFLAL	HLA-C
				(SEQ ID NO:566)	Clone 10
GAGE	5323.2	5,648,226	15 July 1997	YRPRPRRY	HLA-CW6
				(SEQ. ID NO.:567)	
BAGE	5310.1	TBA	TBA	MAARAVFLALSAQLLQARLMKE	HLA-C

Preferred peptide antigens are those of tumor associated antigens (TAA) and chronic infections. Particularly preferred peptide antigens are derived from tyrosinose, gp100 or Melan A for the treatment of melanoma.

The peptide antigens of this invention are readily prepared using standard peptide synthesis means known in the art. Generally they can be prepared commercially by one of numerous companies that do chemical synthesis. An example is American Peptides, Inc., where the distributor is CLINALFA AG (Laufelfingen, Switzerland). The antigens are prepared in accordance with GMP standards. Purity is assessed by analytical HPLC. The product is characterized by amino-acid analysis and tested for sterility and the absence of pyrogens.

In delivering an appropriate antigen, e.g., a polypeptide, to the animal's system it may be delivered directly as the polypeptide, or it may be delivered indirectly, e.g., using a DNA construct or vector, or a recombinant virus that codes for the desired antigen. Any vector driving expression in a professional antigen presenting cell is suitable for this purpose. In the indirect delivery, the antigen is expressed in the cell, to be presented by the MHC Class I on the surface of the cell to stimulate the CTL response.

In a preferred embodiment of the invention an encoded antigen can be delivered in the form of a naked plasmid expression vector. Particularly useful constructs are disclosed in U.S. Patent Application No. 09/561,572 entitled EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS which is incorporated herein by reference in its entirety. The feasibility of and general procedures related to the use of naked DNA for immunization are described in U.S. Patent Nos. 5,589,466, entitled "INDUCTION OF A PROTECTIVE IMMUNE RESPONSE IN A MAMMAL BY INJECTING A DNA SEQUENCE" and 5679647, entitled "METHODS AND DEVICES FOR IMMUNIZING A HOST AGAINST TUMOR-ASSOCIATED ANTIGENS THROUGH ADMINISTRATIONS OF NAKED POLYNUCLEOTIDES WHICH ENCODE TUMOR-ASSOCIATED ANTIGENIC PEPTIDES" which are herein incorporated by reference in their entirety. However the former teaches only intramuscular or intradermal injection while the latter teaches only administration to skin or mucosa. Administration directly to the lymphatic system is greatly more efficient (see examples 6-9, below). Single bolus injection into lymph

node required only 0.1% of the dose required in order to obtain a similar level of CTL response by intramuscular (i.m.) injection. It is therefore feasible to establish a protective response against systemic viral infection with a single bolus delivered i.ln., but not with a dose nearing the practical limit delivered i.m. Even repeated bolus injections i.m. failed to establish a protective response against a peripheral virus infection or transplanted tumor whereas lower doses administered i.m. were completely effective.

In another embodiment of the invention an encoded antigen can be delivered in the form of a viral vector. A wide array of viruses with modified genomes adapted to express interposed reading frames but often no, or at least a reduced number of, viral proteins are known in the art, including without limitation, retroviruses including lentiviruses, adenoviruses, parvoviruses including adeno-associated virus, herpesviruses, and poxviruses including vaccinia virus. Such viral vectors facilitate delivery of the nucleic acid component into the cell allowing for expression. A subset of these vectors, such as retroviruses and parvoviruses, also promotes integration of their nucleic acid component into the host genome, whereas others do not.

Bacteria can also serve as vectors, analogously to viruses, i.e. they can be used to deliver a nucleic acid molecule capable of causing expression of an antigen. For example, a strain of *Listeria monocytogenes* has been devised that effects its own lysis upon entering the cytosol of macrophages (its normal target), thereby releasing plasmid from which antigen was subsequently expressed (Dietrich, G. et al. *Biotechnology* 16:181-185, 1998 which is herein incorporated by reference in their entirety). *Shigela flexneri* and *Escherichia coli* have been similarly used (Sizemore, D.R. et al. *Science* 270:299-302, 1995, and Courvalin, P. et al. *Life Sci.* 318:1207-1212, 1995, respectively, which are herein incorporated by reference in their entirety).

The use of microbial vectors for nucleic acid delivery can be complicated by the immune reactions the vectors themselves provoke. When prolonged or repeated administration is required, antibody elicited by the earlier treatment can prevent useful quantities of the vector from ever reaching its intended host. However, by direct administration into, for example, a lymph node, the combination of proximity to host cells and the much reduced effective dose makes it possible to administer a dose capable of evading or overwhelming an existing antibody titer.

The word vector has been used, here and elsewhere, in reference to several modalities and variously modified (e.g. expression vector, viral vector, delivery vector, etc.). The underlying principle here is that a nucleic acid capable of causing expression of an antigen ultimately arrives in an APC, rather than the antigen itself. Unless modified, explicitly or by local context, wherever the term vector is used herein, it is intended to encompass all such possibilities.

These foregoing techniques are distinct from the approach of modifying the microbial genome (including extra-chromosomal DNA) so that the antigen is produced as a component of the microbe (virus, bacteria, fungi, protazoan, etc., etc.), which is then itself administered as the immunogen. Obviously, the genomic modification would most likely involve the use some reagent falling within the above definition of vector. Again, the distinction is whether the vaccine includes the already synthesized antigen, or a nucleic acid capable of causing an APC to express the antigen in vivo. This strategy constitutes a further embodiment of the invention. For rhetorical clarity we have discussed these approaches as if they were mutually exclusive, but in fact combinations are possible, e.g., a virus vector as above that also incorporates a target epitope into a capsid or envelope protein.

Similarly, antigen presenting cells can also be manipulated in vitro and then themselves used as the active component of a vaccine. Antigen expression can be conferred by delivering nucleic acid encoded antigen using any of the transduction techniques known in the art, including without limitation electroporation, viral or bacterial transduction, lipid-mediated transduction, and biolistic bombardment. Alternatively the APCs may simply be pulsed with antigen. As with any of the other embodiments of this invention an antigen can be an approximately 8-10 amino acid peptide representing a single epitope, a complete protein, a polypeptide encompassing one or more epitopes (including epitopes originally derived from multiple proteins) or other forms of antigen described above.

Antigens may be used alone or may be delivered in combination with other antigens or with other compounds such as cytokines that are known to enhance immune stimulation of CTL responses, such as, GM-CSF, IL- 12, IL-2, TNF, IFN, IL-18, IL-3, IL-4, IL-8, IL-9, IL-13, IL-10, IL-14, IL-15, G-SCF, IFN alpha, IFN beta, IFN gamma, TGF alpha, TGF beta, and the like. The cytokines are known in the art and are readily

available in the literature or commercially. Many animal and human tumors have been shown to produce cytokines such as IL-4, IL-10, TGF-B that are potent modulators of the immune response and that protect tumors from immune-mediated destruction. The production of IL-4, IL-10 or TGF-B by the tumors may achieve this protective effect by suppressing the induction of cellular immunity, including the elaboration of CTL responses. Alternatively, cytokines that support CTL responses can be exogenously added to help in the balance between induction of anti-tumor cell mediated and non-tumor-destructive humoral responses. Several such exogenous cytokines show utility in experimental mouse vaccination models which are known to enhance CTL responses, including GM-CSF, IFN and IL-2. An effective exogenous cytokine that may be used is GM-CSF. GM-CSF is reported to enhance the expression of the so called "co-stimulatory" molecules, such as B7-1 or B7-2 on antigen presenting cells (APC), which are important players in the symphony of interactions that occur during stimulation of CTL by APC. Moreover, GM-CSF is known to induce activation of APC and to facilitate growth and differentiation of APC, thereby making these important CTL stimulating cells available both in greater numbers and potency.

Delivery of the Antigen

This invention is based in part on the observation that a CTL response is not sustained using standard vaccine techniques. While not wanting to be bound by any particular theory, it is thought that T cells do not have a functional memory that is long-lived. Antibody-mediated B-cell memory, on the other hand, appears to have a long-lived effector memory. Thus, delivering an antigen that produces a CTL response must be done over time to keep the patient's immune system appropriately stimulated to attack the target cells. While it has been suggested that antigens and adjuvants can be prepared as biodegradable microspheres or liposomes, none of these preparations have thus far provided a CTL response that is useful for attacking cancer cells or pathogens on a long term basis. The delivery must be sustained over the desired period of time at a level sufficient to maintain the antigen level to obtain the desired response and that it must be delivered from a reservoir having fluid antigen composition that is introduced so that it reaches the animal's lymphatic system.

Ultimately antigen must find its way into the lymphatic system in order to efficiently stimulate CTL. However, delivery of antigen according to the invention can

involve infusion into various compartments of the body, including but not limited to subcutaneous, intravenous, intraperitoneal and intralymphatic, the latter being preferred. Each of these various points of infusion results in antigen uptake into the lymphatic system. The relative amounts of antigen needed to induce a beneficial CTL response varies according to the different sites of infusion. In general, direct infusion of antigen into the lymph system is deemed to be the most efficient means of inducing a CTL response, but that the material difference between the various routes is not necessarily relevant in terms of the quantity of antigen needed, or, in terms of the operating parameters of the invention. The pump systems of the invention are capable of delivering material quantities of antigen in a range that makes the invention suitable for inducing CTL response through delivery to all compartments of the body. CTL stimulation based on delivery of antigen via various routes will be variable, based on the properties of different antigens, which will reflect factors that influence antigen behavior in the body and its rate of equilibration to (or longevity in) the lymph, such an antigen stability in the body fluid, solubility of antigen in body fluid, binding affinity for HLA and potency as a stimulator of CTL.

It is most efficient, and therefore, preferred, that the introduction is done as directly as possible to the lymphatic system to avoid the destruction of the antigen by metabolism in the body. When introduction of a fluid antigen composition occurs subcutaneously, larger quantities of antigen are needed to assure enough antigen reaches the lymphatic system. Such subcutaneous injection is contemplated by this invention if it can be justified by factors such as cost, stability of the antigen, how quickly the antigen gets to the lymph system, how well it equilibrates with the lymph, and other factors that the attending doctor or specialist will recognize. Subcutaneous delivery will generally require 100 to 1000 times more antigen than direct delivery to the lymph system. It is preferable, therefore, that the antigen composition is introduced through a device for local administration to the lymphatic system, e.g. the spleen, a lymph node, or a lymph vessel. The device for local administration may be positioned outside the patient or implanted into the patient. In either case, the device will have a reservoir to hold the fluid antigen-containing composition, a pump to transfer the composition, and a transmission channel leading from the reservoir to be directed to the preferred region of administration in the patient's body. In either case it is preferably portable.

For the device positioned outside the patient's body (the external device), there are numerous devices used for delivering insulin to diabetic patients that are useful in this invention. Generally these are comprised of a reservoir for holding the antigen composition (instead of insulin), a programmable pump to pump the composition out of the reservoir, a transmission channel or line for transmitting the composition, and a means to introduce the composition into the animal's body to ultimately reach the lymphatic system.

The pump employed may be a roller/peristaltic pump, a syringe pump, a piston/valve pump, a gas pressure pump, or the like that has a power source (generally a battery for portability) that is programmable to deliver the desired level of antigen composition to the patient's body and the lymphatic system. A further discussion of the operation of these pumps may be found "Insulin Pump Therapy" by E. Austenst and T. Stahl, Walter de Gruyter, Berlin, New York (1990), at Chapter 3. A list of pumps available at that time that are useful for this invention are given in Table IV.

More recent versions of these pumps are available from the manufacturers shown.

TABLE IV

Name	Manufacturer/distributor	Weight (g)	Size (mm)
Nordisk Infusor	Nordisk	180	100 x 60 x 20
Betatron I	CPI/Lilly	197	99 x 66 x 20
RW 90 P/RW 91 P/ RW 92	Dahedi/EA Satorius Instruments	110	109 x 42 x 22
MRS 4-Infuser	Disetronic	100	75 x 53 x 19
B-D 1000	Becton-Dickinson	131 7	8 x 57 x20
Nordisk Infusor MK 11	Nordisk	180	113 x 65 x 22
MRS 3-Infuser	Disetronic	100	75 x 53 x 18
A S8 MP	Autosyringe/Travenol	161	102 x 64 x 19
Betatron 11	CPULilly	197	99 x 66 x 20
Minimed 504	Pacesetter/Haselmeyer	106	86 x 21 x 51

Minimed 404 S*	Pacesetter	106	86x21 x5l
MRS I /H-Tron	Disetronic/Hoechst	100	75 x 53 x 18

*not yet commercially available

Particularly useful pumps are the Disetronic H-Tron V 100 Insulin Pump from Disetronic Medical Systems, Burgdorf, Switzerland and the Minimed 507 Insulin Pump from MiniMed Inc., 12744 San Fernando Road, Sylmar, California 91342. The MiniMed is particularly useful in that it allows programming a bolus without looking at the pump through a series of audio tones (settable in either 0.5 or 1.0 unit increments) and allows programming a bolus for delivery over an extended period of time - from 30 minutes to 4 hours. It provides up to 12 basal rates (or profiles) that can be programmed per 24 hours from 0.0 – 25 units/hour in 0. 1 unit increments. The device allows for the temporary increase or decrease of a set basal rate from 30 minutes to 24 hours in 30 minute increments, Other features relating to safety, time display, memory, etc. are available from the manufacturer.

The reservoir for the antigen composition should be large enough for delivery of the desired amount of antigen over time and is easily refillable or replaceable without requiring the user to reinsert the means for introducing the antigen composition to the lymph system.

In preparing the antigen compositions of this invention, a composition (preferably aqueous) is prepared to be compatible with the lymph system and is physiologically acceptable to the animal being treated. In preparing the antigen compositions useful in this invention one considers the physicochemical properties of the antigen such as the isoelectric point, molecular weight, glycosylation or other post-translational modification, and overall amino acid composition. These properties along with any known behavior of the drug in different solutions (e.g. different buffers, cofactors, etc.) as well as its in vivo behavior will help guide the choice of formulation components. One parameter that impacts all the major degradation pathways is the solution pH. Thus, the initial formulations also assess the pH dependence of the degradation reactions and the mechanism for degradation can often be determined from the pH dependence to determine the stability of the protein in each solution. Rapid

screening methods usually involve the use of accelerated stability at elevated temperatures (e.g. 40° C) using techniques known in the art.

In general the antigen compositions useful in this invention will be prepared suitable for parenteral injection, in very small quantities. As such a composition must be free of contamination and have a pH compatible with the lymph system. However, because very small quantities of the antigenic composition will be delivered it need not be the same pH as blood or lymph, and it need not be aqueous-based. For antigens that are less soluble a suitable cosolvent or surfactant may be used, such as dimethyl sulfoxide (DMSO) or PLURONIC brand surfactants. The pH range that is compatible is from about 6.7 - 7.3 and can be prepared using water for injection to meet USP specifications (see Remington: The Science and Practice of Pharmacy, Nineteenth Edition; Chapters 86-88). Generally, a standard saline solution that is buffered with a physiologically acceptable weak acid and its base conjugate, e.g., a phosphate or citrate buffering system, will be the basis of the antigen composition. In some cases, a small amount of an antioxidant may be useful to stabilize the composition and prevent oxidation. Factors to consider in preparing the antigen compositions may be found in the 1994 American Chemical Society book entitled "Formulation and Delivery of Proteins and Peptides" (Acs Symposium Series, No. 567) by Jeffery L. Cleland and Robert Langer (Editor)).

For nucleic acid encoded antigens similar considerations apply, although the variety of physico-chemical properties encountered with polypeptides is absent, so that acceptable formulations will have nearly universal applicability. As seen in examples 6-10, plasmid DNA in standard phosphate buffered saline (PBS) is an acceptable and effective formulation. In some embodiments of the invention, DNA is administered continuously or intermittently at short intervals, from a reservoir worn on, or implanted in, the patient's body. It is preferable that the DNA be maintained in a soluble, stable form at or near body temperature over a period of time measured minimally in days. In such applications where the formulated nucleic acid will be delivered from a reservoir over a period several days or longer, the stability of the nucleic acid at room or body temperature for that period of time, as well as its continued sterility, take on increased importance. The addition of bacteriostatic agents (e.g. benzyl or ethyl alcohol) and chelating agents (e.g. EDTA) is useful toward these ends. Formulations containing

about 1-10% ethyl alcohol, 0-1% benzyl alcohol, 0.25-0.5mM EDTA and a citrate-phosphate buffer of pH 7.4-7.8 generally perform well. Such formulations are also appropriate for bolus injections.

Generally the amount of the antigen in the antigen composition will vary from patient to patient and from antigen to antigen, depending on such factors as the activity of the antigen in inducing a response and the flow rate of the lymph through the patient's system. In general the antigen composition may be delivered at a rate of from about 1 to about 500 microliters/hour or about 24 to about 12000 microliters/day. The concentration of the antigen is such that about 0.1 micrograms to about 10,000 micrograms of the antigen will be delivered during 24 hours. The flow rate is based on the knowledge that each minute approximately about 100 to about 1000 microliters of lymph fluid flows through an adult inguinal lymph node. The objective is to maximize local concentration of vaccine formulation in the lymph system. A certain amount of empirical investigation on patients will be necessary to determine the most efficacious level of infusion for a given vaccine preparation in humans.

To introduce the antigen composition into the lymphatic system of the patient the composition is preferably directed to a lymph vessel, lymph node, the spleen, or other appropriate portion of the lymph system. Preferably, the composition is directed to a lymph node such as an inguinal or axillary node by inserting a catheter or needle to the node and maintaining the catheter or needle throughout the delivery. Suitable needles or catheters are available made of metal or plastic (e.g. polyurethane, polyvinyl chloride [PVC], TEFLON, polyethylene, and the like). In inserting the catheter or needle into the inguinal node for example, the inguinal node is punctured under ultrasonographic control using a Vialon™ Insyte-W™ cannula and catheter of 24G3/4 (Becton Dickinson, USA) which is fixed using Tegadenn transparent dressing Tegaderm™ 1624, 3M, St. Paul, MN 55144, USA). This procedure is generally done by an experienced radiologist. The location of the catheter tip inside the inguinal lymph node is confirmed by injection of a minimal volume of saline, which immediately and visibly increases the size of the lymph node. The latter procedure allows confirmation that the tip is inside the node and can be performed to ensure that the tip does not slip out of the lymph node can be repeated on various days after implantation of the

catheter. In case the tip did in fact slip out of location inside the lymph node, a new catheter can be implanted.

In another embodiment, the antigen is delivered to the lymphatic system through an article of manufacture that is implanted in the animal, preferably at or near a site of a lymphatic organ. The article will include a pump that can deliver the antigen at a controlled rate over a pre-determined period of time and is suitable for use in the host. Several devices are known in the art for the delivery of agents (such as drugs) in humans or animals and these can be used or adapted for use in the present invention.

The implantable device will be similar to the external device discussed above in that it comprises a reservoir of a physiologically-acceptable, aqueous, antigen-containing composition that is capable of inducing a CTL response in an animal, a pump positioned in association with the reservoir to deliver the composition at a defined rate, a transmission channel to discharge the composition from the reservoir, and optionally a delivery line connected to the transmission channel, which delivery line is of a size suitable for positioning in the animal and for delivery of the composition in a manner that reaches the lymphatic system of the animal.

Preferably the pump in the implantable device is an osmotic pump of the type used in the ALZET® model device or the DUROS™ model device pioneered by Alza Corporation, Palo Alto, CA or in a device made by Pharmetrix and exemplified in U.S. patent 4,838,862. The osmotic pump utilizes the osmotic effect using a membrane permeable to water but impermeable to a solute. Osmotic pressure built up in a device is used to deliver a composition at a controlled rate over time. A review by Giancarlo Santus and Richard Baker of "Osmotic Drug Delivery: A Review of the Patent Literature" in the Journal of Controlled Release 35 (1995) 1-21, provides useful guidelines for the type of osmotic pumps that are useful in this invention. The osmotic pump forces the composition through a discharge orifice to discharge the composition, Optionally a delivery line connects to the discharge orifice to position the line suitably for delivery to the lymphatic system of the animal. Patents that describe devices useful in this invention include the following U.S. patents: (A) 3,604,417 assigned to American Cyanamid; (B) 4,838,862; 4,898,582; 5,135,498; 5,169,390; and 5,257,987 all assigned to Pharmetrix, (C) 4,340,048; 4,474,575; 4,552,651; 4,619,652; 4,753,651; 3,732,865; 3,760,804; 3,760,805; 3,929,132; 3,995,632; 4,034,756; 4,350,271;

4,455,145; 5,017,381; 5,023,088; 5,030,216; 5,034,229; 5,037,420; 5,057,318; 5,059,423; 5,110,596; 5,110,597; 5,135,523; 5,137,727; 5,174,999; 5,209,746; 5,221,278; 5,223,265; 3,760,984; 3,987,790; 3,995,631; 4,203,440; 4,286,067; 4,300,558; 4,304,232; 4,340, 054; 4,367,741; 4,450,198; 4,855,141; 4,865,598; 4,865,845; 4,872,873; 4,929,233; 4,963,141; 4,976,966, all assigned to Alza Corp. Each of the foregoing patents are incorporated herein by reference.

A basic osmotic pump device incorporates a housing containing a chamber for storing the antigen containing composition to be delivered, separated from a compartment containing an osmotic salt material by a barrier that is moveable under pressure such as a piston or a flexible impermeable membrane. The compartment containing the osmotic salt is separated from osmotic fluid by a semipermeable membrane. In some embodiments, a fluid barrier, such as a foil sheet, isolates the osmotic salt chamber from the osmotic fluid, keeping the pump inactivated until removal of the barrier immediately prior to use. Other osmotic pump devices use body fluid as the osmotic fluid. In these devices a semipermeable membrane separates the osmotic salt compartment from body fluids, and the pump is activated once inserted into the body under exposure to body fluids. In either case, volumetric expansion of the osmotic salt compartment drives the expulsion of the stored antigen from the compartment and into the surrounding environment of the body. These pumps have been highly successful at achieving steady-state pumping and delivery of agents. The pumps are of a small size that can be inserted into a patient, with flexible consideration as to location. This is important in the case of CTL vaccines, since the inventor has determined that efficient induction of CTL responses is contingent on the antigen or antigen expression system being delivered into the lymphatic system, in order to ultimately achieve antigen delivery into a lymphatic organ such as the spleen. Antigen delivered into a lymph node is 100-1000 times more efficient at inducing CTL responses compared with conventional subcutaneous delivery. A modification to the osmotic pump incorporates a microcatheter attachment (i.e., the optional delivery line) at its discharge end, such that when the pump is implanted proximal to a lymphatic organ, such as a lymph node, the catheter can be inserted into the organ to facilitate delivery of the vaccine directly into the lymphatic system.

Prior to the administration of the antigen using any of the above vehicles, methods may be used to assist in the determination of the optimum location for the antigen delivery. For example, when using the osmotic pump, radiography may be used to image a patient's lymphatic flow, to determine where relatively high lymphatic drainage occurs, in order to decide upon an insertion position for the osmotic pump that maximizes delivery into the lymphatic system. Since each patient has unique lymphatic drainage profiles, imaging would be conducted for each individual prior to insertion of osmotic pump for delivery of antigen. When using direct cannulation of the lymphatic vessel, such as in the use of osmotic or insulin pumps to deliver antigen, ultrasound can be used to position the needle directly into the lymphatic vessel and to monitor its positioning over the period of treatment.

The following non-limiting examples are illustrative of the present invention.

EXAMPLES

Materials and Methods For Examples 1-5

Mice: The generation of T cell receptor transgenic mice (TCR+ mice) in which approx. 90% of the CD8+ T cells express a TCR recognizing the immunodominant LCMV-glycoprotein epitope (gp-peptide aa33-41, p33:K.AVYNFATC-SEQ ID NO:569) presented on H-2D^b, has been described in detail. All experimental mice were between 8 and 12 weeks of age and bred and held under strict pathogen free conditions at the Institut Für Labortierkunde at the University of Zurich.

Viruses: LCMV (Armstrong strain) was originally obtained from Dr. M.B.A. Oldstone, Scripps Clinics and Research Foundation, LaJolla, San Diego, CA. Seed virus was grown on BHK cells and plaqued on MC57 cells using an immunological focus assay, as described previously.

Osmotic pump: ALZA model #1007b.

In vivo protection assays for specific CTL activity: The in vivo assay for the detection of CTL activity by challenge infections with LCMV has been described in detail previously (Oehen et al. 1991). Briefly, mice are intravenously challenged with 2X10^3 pfu of LCMV (Armstrong), After 4 days the titer of LCMV is determined using the above mentioned immunological focus assay.

Primary ex vivo cytotoxicity against LCMV-gp: Mice were injected intravenously with 10μg of p33. After 36 hours spleen single cell suspensions were coincubated for 5h with ^{51}Cr-labeled syngeneic EL-4 (H-2^b) target cells, that were either pulsed with p33 or left unpulsed. Specific lysis was calculated as [(experimental ^{51}Cr release - spontaneous ^{51}Cr release) / (total ^{51}Cr release - spontaneous ^{51}Cr release) X 100%].

LCMV induced foot pad swelling reaction: Mice were infected with LCMV (Armstrong) by intradermal injection into the hind footpad (5000 pfu in 30:1), Footpad thickness was measured daily with a spring loaded caliper. Footpad swelling is calculated as (measured thickness - thickness before injection) / (thickness before injection).

Example 1

Continuous release of peptide antigen using osmotic pump induces potent CTL response in C5BL/6 Mice

C57BL/6 mice were either intravenously injected with a single dose of 50μg p33 (including 500 ng GM-CSF) (circles) or were implanted with a micro-osmotic pump releasing a mixture of 50μg of p33 and 500 ng GM-CSF over a time period of 7 days (triangles), or were left naïve (data not shown). After 7 days mice were sacrificed to prepare single cell suspensions from the spleen. Spleen cells were restimulated in vitro for 5 days by p33 pulsed in the presence of low amounts of IL-2. Specific cytotoxicity was measured using ^{51}Cr-labeled EL-4 target cells pulsed with p33. Specific lysis of EL-4 target cells without p33 was less than 16% for all effectors. The results are shown in Figure 1.

Example 2

Continuous release of antigen induces CTL immunity against virus in C57BL/6 mice

C57BL/6 mice were either intravenously injected with a single dose of 50μg p33 (including 500 ng GM-CSF. Pharmingen) or were implanted with a microsomotic pump releasing a mixture of 50μg of p33 and 500 ng GM-CSF over a time period of 7 days, or were left naive. After 7 days specific CTL activity was assessed in vivo using anti-viral

protection assays. C57BL/6 mice were intravenously challenged with LCMV Armstrong strain ($2x10^3$ p.f.u.). After 4 days mice were sacrificed and LCMV titers were determined in spleens using an immunological focus assay. Mice implanted with osmotic pump showed significantly lower virus titers indicating active CTL immunity against the virus (Table V).

TABLE V

C57BL/6 Mice	Virus Titer (loglo)
Single injection	4,2
Single injection	4.6
Single injection	4.0
Pump delivered	2.2
Pump delivered	1.8
Pump delivered	2.0
Unprimed	4.8
Unprimed	3.8
Unprimed	4.4

Example 3

Continuous release of antigen maintains potent CTL effectors in TCR Transgenic Mice

TCR transgenic mice were either intravenously injected with a single dose of 50μg p33 (circles) or were implanted with a microsomotic pump releasing a mixture of 50μg of p33 (triangles), or left naïve (squares). After 36 hours mice were sacrificed to prepare single cell suspensions from the spleen which were assayed ex vivo for p33-specific cytotoxicity using ^{51}Cr-labeled EL-4 target cells pulsed with p33. Similarly mice were either intravenously injected with a single dose of 50μg p33 (circles) or were implanted with a micro-osmotic pump releasing a mixture of 50μg of p33 over a time period of 7 days (triangles), or were left naïve (squares). After 7 days mice were sacrificed to prepare single cell suspensions from the spleen to assay ex vivo p33-specific cytotoxicity using ^{51}Cr-labeled EL-4 target cells pulsed with p33. Specific

lysis of EL-4 target cells without p33 was less than 18% for all effectors. The results are shown in Figures 2A and 2B.

Continuous release of antigen maintains protective CTL response against virus infection.

After 7 days TCR transgenic mice were challenged by intradermal LCMV injection into their hind foot pads ($2x10^3$ pfu in 30µl). The absence of a foot pad swelling reaction, as observed in mice with an implanted pump (triangles), indicates that at the time point of injection there was active CTL immunity inhibiting local replication of the virus in the foot pad. In contrast, foot pad swelling, as observed in mice injected with the peptide as a single bolus (circles) and naive control mice (data not shown), indicated that LCMV successfully replicated in the foot pad in the absence of protective CTL. The results are shown in Figure 2C.

Example 4

Direct delivery of antigen into lymphatic organ dramatically increases efficiency of CTL induction

TCR transgenic mice were injected with graded doses of gp-peptide p33 either subcutaneously (S.C.), intravenously (I.V.) or directly into the spleen (I.S.) via a small abdominal incision. The efficiency of CTL induction was assessed by measuring gp-specific CTL activity 24 hours after injection. CTL activity is known to peak one day after injection of peptide. Mice were sacrificed to prepare single cell suspensions from draining lymph nodes or from spleen to assay ex vivo p33-specific cytotoxicity using ^{51}Cr-labeled EL-4 target cells pulsed with p33. Specific lysis of EL-4 target cells without p33 was less than 12% for all effectors. The results are shown in Figure 3.

Example 5

Dendritic Cells Purified from Mice Receiving Intrasplenic Injection of Peptide Potently Stimulate CTL

The effect of directing peptide delivery into lymphatic system was assessed. Peptide p33 was injected either i.v., s.c. or directly into the spleen of wild-type

C57BL/6 mice. After 2 hours, DCs were isolated from the spleen of animals injected either i.s. or i.v., and additionally from the regional draining lymph nodes of animals injected s.c. Cells isolated from these tissues were sorted for DCs using magnetic beads coupled with a monoclonal antibody recognizing the integrin-alpha chain, a marker specific for DCs in spleen and lymph nodes. The positively and the negatively sorted cell fractions were compared regarding their capacity to in vitro stimulate naive CD8+ T cells from TCR transgenic mice specific for LCMV-gp. Only when peptide had been directly injected into the spleen, the DC containing cell fraction stimulated CTL to proliferate, as measured by ^{3}H-thymidine uptake. This indicated that CTL induction after direct injection of peptide into lymphatic organs reflected efficient loading of DCs with peptide. In contrast, the fraction depleted for DC did not induce proliferation and DCs isolated from lymphoid organs of i.v. and s.c injected mice were not effective stimulators. The results are shown in Figure 4.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Example 6

Induction of CTL response with naked DNA is most efficient by Intra-lymph node immunization.

In order to quantitatively compare the $CD8^{+}$ CTL responses induced by different routes of immunization we used a plasmid DNA vaccine (pEGFPL33A) containing a well-characterized immunodominant CTL epitope from the LCMV-glycoprotein (G) (gp33; amino acids 33-41) (Oehen, S., et al.. *Immunology* 99, 163-169 2000), as this system allows a comprehensive assessment of antiviral CTL responses. Groups of 2 C57BL/6 mice were immunized once with titrated doses (200-0.02μg) of

pEGFPL33A DNA or of control plasmid pEGFP-N3, administered i.m. (intramuscular), i.d. (intradermal), i.spl. (intrasplenic), or i.ln. (intra-lymph node). Positive control mice received 500 pfu LCMV i.v. (intravenous). Ten days after immunization spleen cells were isolated and gp33-specific CTL activity was determined after secondary *in vitro* restimulation. As shown in Fig. 6, i.m. or i.d. immunization induced weakly detectable CTL responses only when high doses of pEFGPL33A DNA (200μg) were administered. In contrast, potent gp33-specific CTL responses were elicited by immunization with only 2μg pEFGPL33A DNA i.spl. and with as little as 0.2μg pEFGPL33A DNA given i.ln. (Fig. 6; symbols represent individual mice and one of three similar experiments is shown). Immunization with the control pEGFP-N3 DNA did not elicit any detectable gp33-specific CTL responses (data not shown).

Example 7

Intra-lymph node immunization is the most efficient way to induce antiviral anamnestic CTL responses.

Similar thresholds for CTL detection were observed when a different readout system was used. Groups of 2 C57BL/6 mice were immunized once with titrated doses of pEFGPL33A DNA (0.2-200μg) and positive control mice received 500 pfu LCMV i.v., as above. Ten days later they were challenged with 5 x 10^4 pfu LCMV i.v. Four days after challenge spleen cells were isolated and *ex vivo* CTL activity was assayed. This time point is too early to detect any primary CTL response to LCMV infection in naive mice (Fig. 7, Controls), but it allows the detection of anamnestic CTL responses in mice which have been previously immunized (Fig. 7, LCMV). As before, mice immunized with 200μg intramuscularly showed only weak anamnestic CTL responses following LCMV challenge, which were not detectable when lower immunizing doses of DNA were used (Fig. 7). Those immunized by the i.spl. route showed strong anamnestic CTL responses which titered out at an immunizing dose of 2μg pEFGPL33A DNA, while the i.ln. route of immunization was again more efficient with anamnestic CTL responses detectable when only 0.2μg pEFGPL33A DNA was administered (Fig. 7).

These results from examples 6 and 7 clearly demonstrate that administration of plasmid DNA directly into lymphoid tissues is 100- to 1000-fold more efficient than

intradermal or intramuscular routes for the induction of CTL responses. In addition, they show that the intra-lymph node route is around 10-fold more efficient than the intrasplenic route.

Example 8

Naked DNA elicits superior protection against systemic and peripheral virus infection by intra-lymph node compared to intramuscular immunization.

To examine whether the enhanced CTL responses elicited following i.ln. immunization with plasmid DNA were able to qualitatively influence antiviral immunity, we used challenge infections with LCMV or with recombinant vaccinia virus expressing the LCMV-G (Vacc-G2) as models of systemic and peripheral virus infection, respectively. When systemic antiviral immunity was assessed by challenging the immunized mice (groups of 3 C57BL/6 mice) with a high dose of LCMV i.v. (500 pfu), mice which had been immunized once with 200μg pEGFPL33A DNA i.m. showed only partial and incomplete protection against systemic LCMV challenge, while those which had received 20μg of pEFGPL33A DNA by the i.spl. or i.ln. routes were completely protected (Fig. 8A).

Eradication of Vacc-G2 infection from peripheral organs such as ovaries, is dependent upon the presence of high levels of recently activated effector $CD8^+$ T cells (Kündig, T.M. *et al. Proc. Natl. Acad. Sci. USA* 93, 9716-9723, 1996; Bachmann, M.F., et al. *Proc. Natl. Acad. Sci. USA* 94, 640-645, 1997). Groups of 3 C57BL/6 mice were immunized four times at 6 day intervals with pEFGPL33A DNA administered either i.m. (100μg per immunization) or i.ln. (10μg per immunization). Five days after the last immunization they were challenged with 5×10^6 pfu Vacc-G2 i.p. and vaccinia titers in ovaries were assessed after a further 5 days. Repeated i.m. immunization with pEFGPL33A DNA had no influence on the growth of Vacc-G2 in peripheral tissues (Fig. 8B). In contrast, mice which were repetitively immunized with pEFGPL33A DNA by the i.ln. route were completely protected against peripheral infection with Vacc-G2 (Fig. 8B).

These results illustrate that although repeated i.m. immunization with naked DNA induced detectable CTL responses, these were never of sufficient magnitude to offer protection against virus infection. In contrast, immunization with 10-fold lower

amounts of DNA directly into lymphoid organs elicited quantitatively and qualitatively stronger CTL responses, which gave complete protection against systemic or peripheral virus challenge.

Example 9

Intra-lymph node DNA immunization elicits anti-tumor immunity

To examine whether the potent CTL responses elicited following i.ln. immunization were able to confer protection against peripheral tumors, groups of 6 C57BL/6mice were immunized three times at 6-day intervals with 10μg of pEFGPL33A DNA or control pEGFP-N3 DNA. Five days after the last immunization small pieces of solid tumors expressing the gp33 epitope (EL4-33) were transplanted s.c. into both flanks and tumor growth was measured every 3-4d. Although the EL4-33 tumors grew well in mice that had been repetitively immunized with control pEGFP-N3 DNA (Fig. 9), mice which were immunized with pEFGPL33A DNA i.ln. rapidly eradicated the peripheral EL4-33 tumors (Fig. 9).

Example 10

Differences in lymph node DNA content mirrors differences in CTL response following intra-lymph node and intramuscular injection.

pEFGPL33A DNA was injected i.ln. or i.m. and plasmid content of the injected or draining lymph node was assessed by real time PCR after 6, 12, 24, 48 hours, and 4 and 30 days. At 6, 12, and 24 hours the plasmid DNA content of the injected lymph nodes was approximately three orders of magnitude greater than that of the draining lymph nodes following i.m. injection. No plasmid DNA was detectable in the draining lymph node at subsequent time points (Fig. 10). This is consonant with the three orders of magnitude greater dose needed using i.m. as compared to i.ln. injections to achieve a similar levels of CTL activity. $CD8^{-/-}$ knockout mice, which do not develop a CTL response to this epitope, were also injected i.ln. showing clearance of DNA from the lymph node is not due to $CD8^{+}$ CTL killing of cells in the lymph node. This observation also supports the conclusion that i.ln. administration will not provoke immunopathological damage to the lymph node.

Example 11

Stability of plasmid in different formulations.

DNA is a relatively stable molecule in the kind of formulations of interest to test and thus little loss of material would be noted if the total amount of DNA were to be measured. Instead, the ratio of supercoiled to open-circle DNA was measured. Since a single nick anywhere in either strand of the DNA molecule will allow a supercoiled plasmid to relax to an open circle conformation this is an exquisitely sensitive indication of damage to the DNA backbone. Plasmid was formulated, placed in vials in triplicate and incubated at 37°C. After 1, 3 and 7 days aliquots were removed, subjected to anion exchange HPLC, and the peak areas corresponding to supercoiled and open-circle DNA compared (see fig.11). Nine formulations were tested:

1. 10% Ethanol, 0.25mM EDTA, Citrate Phosphate pH 7.6
2. 10% Ethanol, 0.25mM EDTA, Citrate Phosphate pH 7.4
3. 1% Ethanol, 0.5mM EDTA, Citrate Phosphate pH 7.4
4. 1% Ethanol, 0.5mM EDTA, 1X PBS pH 7.4
5. 0.5% Benzyl Alcohol, 0.25mM EDTA, Citrate Phosphate pH 7.6
6. 1% Benzyl Alcohol, 1% Ethanol, 0.5mM EDTA, Citrate Phosphate pH 7.6
7. 1% Benzyl Alcohol, 1% Ethanol, 0.5mM EDTA, 0.1M TRIS pH 7.4
8. 1% Benzyl Alcohol, 1% Ethanol, 0.5mM EDTA, 0.1M TRIS pH 8.2
9. 1% Benzyl Alcohol, 1X PBS pH 8.2

Citrate Phosphate Buffer pH. 7.4 was made by mixing 9.15 parts (by volume) of 0.1M citric acid with 90.85 parts of 0.2M Sodium Phosphate Dibasic. Citrate Phosphate Buffer pH. 7.6 was made by mixing 6.35 parts (by volume) of 0.1M citric acid with 93.65 parts of 0.2M Sodium Phosphate Dibasic. These solutions were then added to the other components to create a 2x buffer which was mixed with a equal volume of DNA in water. Thus the final concentrations of citrate and phosphate in the above buffers was on the order of 3mM and 90mM, respectively.

Formulations 1-3 and 6 gave superior results (see fig. 11).

Example 12

Stability of formulated plasmid in operating MINIMED 407C infusion pumps.

Using a modification (final concentrations of 0.1 M sodium phosphate dibasic and 0.05 M citric acid; pH 7.6±0.2) of formulation 6 above, aliquots of 80, 160, and 320 µg DNA/ml were prepared and loaded in triplicate into MINIMED 3.0 reservoir syringes. A 200µl sample was dispensed and the reservoir syringes were inserted into MINIMED 407C infusion pumps and assembled with SILHOUETTE infusion sets fitted with 3.1mm catheters. The pump assemblies, set to dispense 10µl/hour and with the catheters inserted into collection vials, were placed in 37°C incubators. At 4 and 8 days the catheters were briefly detached and 200 µl bolus samples dispensed directly from the reservoir. The concentration of supercoiled DNA was determined for each sample by anion exchange HPLC and the use of a standard curve constructed with known concentrations of DNA. Plotting the resultant concentrations versus time allows one to derive a slope indicating the rate of loss of supercoiled DNA. The average (of the triplicate samples) rates of loss were –0.056±1.88, 0.24±1.01, and 0.048±0.49 µg DNA/day for the 80, 160, and 320 µg DNA/ml samples, respectively. None of these differ significantly from zero.

Example 13

Administration of a DNA plasmid formulation of a therapeutic vaccine for melanoma to humans.

SYNCHROTOPE TA2M, a melanoma vaccine encoding HLA-A2-restricted tyrosinase epitopes was formulated in 1% Benzyl alcohol, 1% ethyl alcohol, 0.5mM EDTA, citrate-phosphate, pH 7.6. Aliquots of 80, 160, and 320 µg DNA/ml were prepared for loading into MINIMED 407C infusion pumps. The catheter of a SILHOUETTE infusion set is placed into an inguinal lymph node visualized by ultrasound imaging. The assembly of pump and infusion set was originally designed for the delivery of insulin to diabetics and the usual 17mm catheter has been substituted with a 31mm catheter for this application. The infusion set is kept patent for 4 days (approximately 96 hours) with an infusion rate of about 25 µl/hour resulting in a total infused volume of approximately 2.4 ml. Thus the total administered dose per infusion will be approximately 200, 400, and 800 µg, respectively, for the three concentrations

described above. Following an infusion subjects will be given a 10 day rest period before starting a subsequent infusion. Given the continued residency of plasmid DNA in the lymph node after administration (as in example 10) and the usual kinetics of CTL response following disappearance of antigen, this schedule will be sufficient to maintain the immunologic CTL response.